

EFFECT OF AGRONOMIC PRACTICES ON DISEASE INCIDENCE, SEVERITY,
AND IMPACTS IN MONTANA CROPPING SYSTEMS

by

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ABSTRACT

Integrated pest management is at the foundation of sustainable cropping systems. This thesis investigated 1) the influence of alternative host plants and agronomic practices on *Wheat streak mosaic virus* (WSMV) risk, and 2) how cover crop termination methods influence diseases in grazed organic, tilled organic, and chemical no-till systems. To assess the influence of alternative hosts including volunteer wheat, *Bromus tectorum*, *Setaria viridis*, and *Zea mays* on WSMV incidence and its vector, the wheat curl mite (*Aceria tosichella*, WCM) movement during the fall, a ‘trap plant’ capture system was used. In 2013, alternative hosts had similar WCM infestation levels compared to the control in most weeks. In 2014, spring planted *B. tectorum* and volunteer wheat increased the incidence of WSMV and abundance of WCM compared to control. In a study assessing the impact of planting date and winter wheat variety on WSMV incidence, there was almost no infection of WSMV across resistant wheat varieties. However, Pronghorn, a susceptible wheat variety, had a higher WSMV incidence at the early planting date than recommended and late planting dates. In a companion study of the impact of wheat variety and timing of N application on WSMV incidence, results did not differ across N application timing across resistant varieties. However, early spring N application in 2014 had a higher WSMV incidence compared to fall and late spring N application in Pronghorn and Yellowstone. A study assessing the impact of cropping systems on diseases indicated that disease incidence during the transition to organic period in 2013 and 2014 as well as an established organic year, 2016, was similar at tillering and flowering stages of winter wheat between the grazed organic, tilled organic, and chemical no till system. However, disease incidence was variable between systems at the matured growth stage in 2014 and 2016. In 2015, disease incidence varied between systems at all growth stages. Overall, disease severity was similar in winter wheat between grazed organic, tilled organic, and chemical no till systems, indicating disease is not a major constraint to organic methods of crop production during the transition period.

CHAPTER ONE

BACKGROUND, LITERATURE REVIEW AND RESEARCH OBJECTIVES

Introduction

Sustainable disease management is an integral component of global food security (Lucas, 2011; Strange and Scott 2005) and integrated management is at its foundation. Plant diseases cause about 16 – 18 % of crop losses globally and threaten the food security (Oerke 2006). In order to reduce the losses they cause, it is necessary to develop effective long term disease management strategies. Disease management is difficult due to existence of multiple interaction among components of a patho-system including host, pathogen, and environment as well as, a vector for some patho-systems (Jones 2014). The interactions of all these components should be jointly considered in its assessment (Campbell and Madden 1990; De Wolf and Isard 2007; Jones 2014).

The efficacy of disease management is many times constrained by the lack of integration of multiple and dynamic approaches (Zhan et al. 2014). From the viewpoint of a reductionist or therapeutic approach to pest management, plant disease is the product of a particular pathogen infecting a plant (Lewis et al. 1997). Control measures are therefore primarily focused on individual pathogens. Within this context, research has been directed to discover and develop the use of bio-chemistry, molecular biology, and genetics for disease management (Berenbaum et al. 2000; National Academy of Science, Engineering, and Medicine 2016; Scott et al. 2016). These tactics when applied as a sole means of control resulted in short-term relief (Zhan et al. 2014; Lewis et al. 1997).

Therefore, preventive disease management strategies that are multidisciplinary and dynamic focusing on underlying interacting components of the disease in a system can provide long-term sustainable disease management (Lewis et al. 1997). Due to the variability of plant pathogens and their dynamics in time, space, and genetics (Stange and Scott 2005) as well as interactions occurring among components of disease cycle and across trophic levels (Hedlund et al. 2004; Schroter et al. 2004), long-term solutions can be obtained by preventive measures addressing “pests” as an inherent component of agricultural systems rather than a single entity.

Literature Review

Abiotic and Biotic Stresses Affecting Wheat Production in the Northern Great Plains

Wheat (*Triticum aestivum* L.) is one of the main crops produced in the Great Plains states of the USA including Montana, Wyoming, North Dakota, South Dakota, Colorado, Nebraska, Kansas and Texas. Wheat production in the Northern Great Plains (NGP) is regularly affected by several abiotic and biotic stressors threatening its production. Variable weather and climatic conditions, prolonged droughts, and nutrient limitations are common abiotic stresses of wheat fields (Havlin et al. 2005). Along with these abiotic stresses, number of biotic agents such as bacterial, fungal, and viral pathogens are major cause of losses to wheat production (Appel et al. 2015). It is difficult to make an accurate estimate of crop loss, but researchers in Kansas estimated the cumulative disease loss for wheat crop from 1976 to 2015 was ranged from 0.2 % and 22.4 % of potential yields; in 2015 the cumulative loss of wheat crop was 22.2 % (78 kg

ha⁻¹) of potential yields of crop without diseases (Appel et al. 2015). Hence, wheat disease management is crucial to secure wheat yield.

Disease management in wheat production systems is complex. A typical disease cycle of a pathogen consists of interaction between a plant host, a pathogen and the environment (Francl 2001). All three components must be present in order to have disease. Changes in characteristics of one component can positively or negatively affect the degree of disease severity (Agrios 2005). This complexity is further increased when other components like the biological characteristics of the vectors and alternative hosts that are involved in the disease cycle. An example of a complex host-vector-pathogen system involving alternative hosts is the wheat-vector-virus complex.

Viral pathogens are major threat to wheat production throughout the Great Plains. The most common viral pathogens of wheat are, *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), *Wheat mosaic virus* (WMoV), formerly known as High Plains Virus, *Barley yellow dwarf virus- PAV* (BYDV-PAV), and *Cereal yellow dwarf virus -RPV* (CYDV-RPV) (Burrows et al. 2015). Kansas researchers estimated 1.2 %, 1 % and 2.7 % losses annually due to wheat cereal viruses in 2013, 2014, and 2015 respectively (Appel et al. 2015). However, even the estimation of a 1 % yield loss turns into loss of million dollars to the total wheat production (Burrows et al. 2015). These viral pathogens are transmitted by vectors within and between fields. For example, BYDV is transmitted by different aphid species (D'Arcy and Domier 2000), while WSMV, TriMV and WMoV are transmitted by an eriophyid mite, wheat curl mite (*Aceria tosichella* Keifer).

Wheat streak mosaic virus

Wheat streak mosaic virus is the type species of the genus *Tritimovirus* within the family *Potyviridae* (Stenger et al. 1998). WSMV is a single stranded positive-sense RNA virus having 700 nm filamentous particles with a 15 nm diameter (Carroll et al. 1982). It is a common viral pathogen of cereal crops in the Great Plains of USA and causes sporadic epidemics worldwide (Burrows et al. 2015; Ellis et al 2003; Navia et al. 2013). WSMV can infect a variety of hosts including wheat, barley (*Hordeum vulgare* L.), rye (*Secale cereal* L.), corn (*Zea mays* L.), foxtail millet (*Setaria italic* (L). P. Beauvois), pearl millet (*Pennisetum glucum* (L.) R. Br.), and oat (*Avena sativa* L.). Along with crop hosts, WSMV infect many grass weedy species including downy brome (*Bromus tectorum* L.), rye brome (*B. secalinus* L.), green foxtail (*S. viridis* (L.) Beauv), jointed goat-grass (*Aegilops cylindrica* Host), and barnyard grass (*Echinochloa crus-gali* (L.) Beauv.) (Brey et al. 1998; Ito et al. 2012; Somsen and Sill 1970). These grass species play significant role in the epidemiology of the virus (Christian and Willis 1993; Slykhuis 1955) as the grass species can serve as virus and vector reservoirs in absence of wheat host and become source of infection when next season wheat emerged in the field (Brey et al. 1998; Somsen and Sill 1970).

Wheat streak mosaic virus causes reduction in chlorophyll content creating yellow streaked leaves with a mosaic pattern (Brakke 1987; Rahman et al. 1974). Other symptoms of WSMV include stunted growth, reduced root biomass, and decreased water use efficiency (Price et al. 2010). These reductions of root biomass and water use efficiency in turn results grain yield and quality reduction. In the Great Plains, yield

losses range from 13 to 70 percent due to WSMV infection in mechanically inoculated trials (Rahman et al. 1974; Sharp et al. 2002; Miller et al. 2014). However, a yield loss caused by naturally infected WSMV in Kansas is estimated at an average of 1 to 2 percent annually (Appel et al. 2015).

Wheat Curl Mite

Wheat curl mite is an eriophyid soft bodied, spindle shaped mite with two pairs of legs (Keifer et al. 1982). The mite measures 210 - 250 microns and is difficult to detect in the field without magnification. The WCM life cycle consists of egg, two nymphal stages and an adult stage with two quiescent periods before the final molt (Manson and Oldfield 1996). Wheat is the main host of WCM and feeding by the mite causes curling of the leaf edges (Slykhuis 1955). The curling of the leaf creates a protective and favorable microclimate for the growth and reproduction of the WCM (Thomas et al. 2004). WCM has an enormous reproductive potential and one plant head may contain an average of 18,000 WCM in highly infected fields (Harvey et al. 1990). The feeding of aviruliferous mites can cause up to 15 - 20 % of yield reduction (Harvey et al. 2000). However, the most significant loss associated with WCM is due to WSMV infection.

The WCM is the only known vector of WSMV and transmits the virus in a semi-persistent or circulative, non-propagative manner (Slykhuis 1955; Siriwetwivat 2006). The virus is present in the foregut of the WCM and transmission efficiency increases with prolonged feeding. The retention period lasts from hours to days (Stenger et al. 2005). WSMV particles have also been found in haemocoel and in the salivary gland of WCM,

suggesting circulative or persistent types of transmission (Oldfield and Proeseler 1996; Paliwal 1980; Siriwetwivat 2006). WSMV is not transmitted by regurgitation or transovarially (Paliwal 1980). Transmission efficiency varies by the growth stage, where nymphal stages are more efficient at transmitting WSMV than adults (Siriwetwivat 2006).

Two types of WCM have been identified based on genetic sequence within the mitochondrial cytochrome oxidase subunit I and cytochrome oxidase subunit II regions of WCM ribosomal DNA (Hein et al. 2012). Wheat curl mites are categorized as Type 1 (Kansas, Montana, South Dakota and Texas) and type 2 (primarily found in Nebraska). Mixtures of both WCM biotypes can be found within individual fields in different regions throughout the Great Plains (Siriwetwivat 2006).

WCM is a species complex of multiple, genetically distinct cryptic lineages with variable host ranges (Carew et al. 2009; Skoracka et al. 2012). WCM and WSMV both need living host to survive. They survive in several grassy alternative hosts during fallow periods. Therefore, existing of a genetically distinct cryptic species might be due to the host-range diversity (biological strains) and/or environmental conditions (Slykhuis 1955) or due to coevolution in hosts including wheat and other grasses (physiological strains) (Del Rosario and Sill 1965). Different biotypes and lines of WCM differ in their ability to transmit different species of viruses. Seifers et al. (2002) and McMechan et al. (2014) reported that Type 2 genotype transmits TriMV and WMoV more efficiently than the Type 1 genotype. These genotypes differ in their ability to transmit viruses from singly infected or co-infected with two viruses. For example, Type 2 WCM transmits WSMV at higher rates than it co-infected with TriMV (Oliveira-Hofman et al. 2015).

Wheat Streak Mosaic Disease Management

There is no effective chemical option available for the control of WSMV or WCM (Harvey et al. 1979; Murphy 2016) and preventive cultural practices are the main methods of management. These include control of alternative host plant species, altering planting date, use of resistant varieties, and modification of nitrogen (N) application in the fields (Hadi et al. 2011; Jiang et al. 2005; Miller et al. 2015). Volunteer wheat along with other grasses acts as a “green bridge” to spread the WCM and WSMV from one growing season to the next (Jiang et al. 2005). Control of these green bridge hosts and delayed plating date are the most commonly used methods of WSMV management (Somsen and Sill 1970).

Control of Alternative Host Plants for

Wheat Streak Mosaic Management: Management of the WSMV is complicated by its broad range of alternative or reservoir hosts that act as a “green bridge” from wheat harvest to the emergence of newly planted winter wheat (Lu et al. 2011; Navia et al. 2013; Zhang et al. 2015). Viruliferous mites move from infected wheat fields before and during senescence to the alternative hosts where they survive and reproduce (Slykhuis 1955; Jiang et al. 2005). After emergence of wheat, WCM can move from alternative hosts to the newly emerged wheat and transmit virus. Risk of WCM infestation on newly emerged wheat increase by hail events as well as cool and moist fall. Hail events drop out the seeds from maturing wheat heads and these seeds can then germinate in moist soil conditions and are known as ‘volunteer’ wheat. Control of alternative hosts to break the “green bridge” is an effective management tactic for WSMV. However, the efficacy of

commonly used methods of “green bridge” management such as herbicide and tillage depend upon farm management and weather conditions and both methods have ecological shortcomings like soil erosion, air and water pollution, resistance, pesticide trademill, pest resurgence (Geer et al. 2006; Logan et al. 1991; Powles and Yu 2010; Guedes et al. 2016). In addition, plants may take from days to weeks to die following an herbicide application, allowing time for the WCM to move to susceptible host plants downwind of the inoculum source (Thomas et al. 2004; Jiang et al. 2005). WCM can survive outside the host for up to a week depending upon temperature and relative humidity (Wosula et al. 2015). Therefore, herbicides and tillage should be applied well before planting to reduce the risk of WCM infestation of the new crop (Thomas et al. 2004; Jiang et al. 2005). The knowledge of the peak period of WCM infestation on alternative hosts and timing of WCM migration to the newly emerged crop can provide a better understanding of disease risk in an area.

Impact of Planting Date for

Wheat Streak Mosaic Management: In the WSMV disease cycle, spread of the virus depends on the movement and mobility of the WCM. There are two critically important components of disease cycle of WSMV in the fall: first, successful dispersal of WCM from maturing wheat to alternative hosts that allow for WCM and WSMV to survive; and second, reproduction of WCM on those alternate hosts to sustain the population until the new crop emerges and their dispersal to newly emerged crops. Altering planting date is a commonly used management tactic to reduce the WCM infestation and WSMV infection (Brakke 1987; Wills 1984). Risk of mite infestation and

virus infection depends upon presence of alternative hosts and mite movement during fallow period. For example, planting wheat during the period of reduced WCM movement is an effective approach to reduce the risk of disease incidence (Slykhuis et al. 1956). Early seeding of winter wheat increases the duration of crop exposure to WCM and WSMV during and just after the harvesting period of cereal grain crops (Wegulo et al. 2008). Additionally, the infection prior to tillering stages had severe impact than the WSMV infection on later stage (Hunger et al. 1992).

Use of Resistant Varieties for
Wheat Streak Mosaic Management: Host resistance is an effective tool for

WSMV management (Harvey et al. 1994; Holtzer et al. 1996; Zhang et al. 2015). Wheat cultivars resistant to WSMV have been developed (Fahim et al. 2012; Friebe et al. 2011; Martin et al. 2007; Zhang et al. 2015). Three WSMV resistant genes; *Wsm1*, *Wsm2* and *Wsm3* have been identified and transferred onto wheat (Friebe et al. 2011; Graybosch et al. 2009; Lu et al. 2011; Seifers et al. 2007; Zhang et al. 2015) but, only a few WSMV resistant wheat varieties such as 'Mace', 'Snowmass' and 'RonL' are available.

Moderately resistant varieties including TAM 111 and TAM 112 are also available (Friebe et al. 1991; Graybosch et al. 2009; Lu et al. 2011). However, resistant varieties are not available everywhere the disease occurs and have been proven to be a temporary solutions as resistant genes for WSMV are not effective at high temperatures (above 24° C - 27° C) (Seifers et al. 2006, 2007).

Resistance to the WCM has been developed, but just as WSMV, vector resistance is also difficult to maintain (Malik et al. 2003). Curl mite colonization (*Cmc*) genes have

been identified and transferred into wheat. The *Cmc1* and *Cmc4* genes were identified from Tauschi's goat-grass (*Aegilops tauschii* syn. *Triticum tauschii* (Cox et al. 1999; Malik et al. 2003; Thomas and Conner 1986). The *Cmc2* gene was identified from tall wheat grass (*Agropyron elongatum* (Host). Beauv. (Chen et al. 1999; Whelan and Hart 1988), and the *Cmc3* gene was identified in the rye (*Secale cereal* L.) (Harvey and Livers 1975). TAM 107 has been used as mite resistant variety throughout the southern High Plains and effectively reduced the incidence of WSMV than cultivars with no mite resistance (Harvey 1994). However, later it was determined that a strain of WCM had overcome the resistance conferred by *Cmc3* on TAM 107 (Harvey et al. 1997). WCM populations were found to colonize on wheat varieties containing *Cmc1* and *Cmc2* even before they were commercially available (Harvey et al. 1999). The possible reason of overcoming the host resistance is due to the presence of biotypes and several isolates of mites and virus (Robinson and Murray 2013). These issues demonstrate the difficulties in using host plant resistance as a sole control tactic especially under changing environment and vector populations. Understanding the performance of available varieties integrated with other cultural practices could improve WSMV management strategies.

Impact of Nitrogen Application on

Wheat Streak Mosaic Management: Essential nutrient availability, particularly

available N has been increased in recent years to enhance yields and grain protein content in wheat production (Hawkesford 2014; Jones and Olson-Rutz 2012). Along with its impact on yield and grain protein contents, N can influence disease incidence and severity by affecting biochemistry and host quality for vector and pathogen reproduction

(Dordas 2008; Walters and Bingham 2007). Past studies have demonstrated that viral infection is affected by the nutrient type and quantity (Huber and Watson 1974; Borer et al. 2010) and effect of nutrients differed even in closely related virus species (Lacroix et al. 2014). For example, *Barley yellow dwarf* (BYDV-PAV) had higher infection rates with N and phosphorus (P) supply, but infection rates of *Cereal yellow dwarf virus* (CYDV-RPV) decreased with P supply (Lacroix et al. 2014). N concentrations may increase herbivore damage by increasing herbivore preference, performance, and reproduction (Awmack and Leather 2002; Throop and Lerdau 2004). At high N availability, the proportion of young tissues is higher than mature tissues in the plant leaf, and amino acid concentration is also increased on the leaf surface, which may make the plant more susceptible to disease infection (Dordas 2008).

Previous research has reported that the performance and reproduction of insect herbivores is increased with increase host N concentration (Awmack and Leather 2002; Throop and Lerdau, 2004). For example, the population of onion thrips (Buckland et al. 2013), and population of different aphids species (Hosseini et al. 2015; Stafford et al. 2012) is increased with N concentration. Miller et al. (2015) observed that population growth rate of viruliferous WCM increased with N fertilization and WSMV infection rates increased with increasing soil nitrate concentration in winter wheat. Timing of application can be manipulated in addition to the quantities of nutrient applied, but little work has been done in the manipulation of timing of nutrients application for the disease control (Walters and Bingham 2007).

No-till Farming Systems

Intensive reliance on tillage poses threat to sustainable agriculture as it may lead to the unintended consequences such as increased soil erosion (Greer et al. 2006), air and water pollution (Logan et al. 1991; Stoate et al. 2001), and decreases in carbon sequestration (West and Marland 2002), soil moisture, organic matter (Holland 2004), density of beneficial arthropods (Kladivko 2001; Lalonde et al. 2012) and soil micro-organisms (Roper and Gupta 1995). Thus, interest in no-till and conservation tillage has been increased (Carr et al. 2011; Sumner et al. 1981). No-till is suitable for the semi-arid environment of the NGP particularly for water conservation and soil erosion reduction (Tanaka et al. 2010; Peterson et al. 1996). However, disease caused by some saprophytic fungi including *Pythium spp.*, *Rhizoctonia spp.*, *Fusarium spp.*, and *Pyrenophora tritici-repens* can be more problematic under no till system (Bockus and Shroyer 1998; Bailey et al. 2001; Cook and Haglund 1991; Smith et al. 2003; Wright and Sutton 1990).

Disease development and dynamics in a farming system depends upon the agronomic practices applied, crop types, and biology of the pathogen (Bockus and Shroyer 1998). For example, conventional farmers used pesticides for pest management and cover crop termination. Excessive reliance on pesticides has resulted to the selection of pesticide resistance pest biotypes, buildup of pesticide residues in soil and water, non-target effects on beneficial organisms, and pest resurgence (Guedes et al. 2016; Powles and Yu 2010). On the other hand, organic growers solely rely on tillage during fallow periods for weed and cover crop termination. Due to the adverse effect of tillage, there is a growing interest in alternative termination methods that enhance agricultural

sustainability in dryland farming (Carr et al. 2011, 2013). These management practices can impact on disease incidence and severity.

Cover Crops and Termination Methods

Cover crops are suites of plants established for temporary soil cover that can be used to improve soil physical and chemical properties, enhance nutrient cycling (Delgado et al. 2007) and manage pests (Altieri and Nicholls 1999; Gallandt et al. 1999; Sulc and Franzluebbers 2014; Tillman et al. 2012). Organic growers in NGP rely on green manure cover crop to provide N for crops. Legume cover crops can increase available N to subsequent crops due to biological nitrogen fixation (Snapp et al. 2005). Cover crops are used to replace summer fallow that is used for water conservation in dryland farming (Tanaka et al. 2010). Cover crops use soil moisture and may reduce water availability for the subsequent crop; therefore cover crops need to be terminated in a timely manner (Zentner et al. 2004). Tillage or herbicides are the common cover crop termination methods in the NGP (Foster 1990), and both tactics have ecological shortcomings described above.

Livestock integration into annual crop production for the termination of cover crops can be an alternative to tillage or herbicide as it may provide alternative sources of revenue through production of food and fiber (Entz et al. 2002; Franzluebbers 2007; Russelle et al. 2007; Thiessen Martins and Entz 2011). However, livestock integration represents an additional capital investment for growers (Franzluebbers and Stuedemann 2014; Undersander et al. 2002). Targeted grazing with small ruminants such as sheep

provides opportunities for farmers to lease their land and relieve costs associated with livestock integration (Russelle et al. 2007). Furthermore, sheep-crop production integration is well-suited to the NGP as animals are easily transported and available in the region (NASS 2015).

Project Goals and Objectives

The patho-system of WSMV is complex consisting of host, virus, vector, environment, and impact of agronomic practices on their interaction. Management of wheat streak mosaic becomes more complicated by the presence of many virus strains and biotypes of WCM associated with different transmission efficiencies (Lu et al. 2011; Navia et al. 2013; Skoracka et al. 2012; Zhang et al. 2015). Due to the existence of this wheat-mite-virus complex, researches focus on a static single disease management practice could not provide effective, long term solution for WSMV management.

Knowledge of WCM movement to and from alternative hosts and subsequent disease development is essential to understand disease dynamic. Past studies reported that several grassy weeds are susceptible to WCM and WSMV (Connin 1956; Ito et al. 2012; Somen and Sill 1970). However, it is not fully understood at what times between growing seasons WCM populations are most likely to move and the role of alternative hosts on survival and reproduction of WCM during the fall. These factors are important to assess the likelihood of WCM infestation and transmission of WSMV and knowledge of pathogen and vector ecology is primary factor to develop ecologically based management. Additionally, the use of single management practice such as adjusting

planting date or use of a resistant variety could not manage WSMV successfully in the long term. Previous studies have assessed the impacts of planting date, N fertilization, and use of resistant varieties on WSMV incidence using mechanical inoculation of WSMV rather than WCM (Hunger et al. 1992; Miller et al. 2015; Talbert et al. 1996). However, mechanical inoculation may not give accurate estimation of disease risk (Miller et al. 2014). Therefore, for effective long-term management of WSMV, research was needed to focus on to understand the risk factors on spread of WCM and WSMV within and across the fields as well as the impact of integration of cultural agronomic practices on WSMV incidence. This study evaluated the combined effect of planting date and winter wheat variety on WSMV incidence using biological inoculation. The information of virus and mite ecology in regards to change in agronomic practices within field as well as in the surrounding can be used as drivers of epidemics to develop forecasting model for WSMV epidemics under changing climatic scenario. These researches will enable us to develop better ecologically based dynamic management strategies for WSMV in the NGP.

Reducing tillage intensity or herbicide use could increase sustainability of crop production in dryland farming. Health and ecological shortcomings of tillage and herbicides described above demand an alternative approach of cover crop termination methods. Targeted sheep grazing is well suited to the NGP as sheep are easily transported and available in the region (NASS 2015). It is essential to understand how agronomic practices impact plant disease before their implementation in the cropping system. While impact of cover crops and sheep grazing on weed communities, carabid beetle communities, wheat stem sawfly larval mortality have been studied (Barroso et al. 2015;

Miller et al. 2014; McKenzie et al. 2016; Goosey et al. 2005); less is known about their impact on plant disease incidence and severity in dryland farming systems.

Therefore, the study has following goals:

- Assess the role of alternative host on wheat curl mite infestation and *Wheat streak mosaic virus* infection and disease dynamics
- Explore the impact of planting date, N application timing, and crop variety on *Wheat streak mosaic virus* incidence in winter wheat
- Determine the impact of cropping system and cover crop termination methods in diseases of wheat and lentils

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CHAPTER TWO

ROLE OF ALTERNATIVE HOSTS ON WHEAT CURL MITE (*ACERIA TOSCHELLA*
KEIFER) INFESTATION AND WHEAT STREAK MOSAIC VIRUS INFECTION
AND THEIR DYNAMICSIntroduction

Effective and sustainable ecological- and epidemiological-based plant viral disease management strategies should be based on preventive crop protection measures (Jeger et al. 2004; Jones 2014). Understanding the ecology of host, pathogen, and vector, as well as their environmental interactions is important for developing long-term preventive management strategies for vector borne viral diseases. The analysis of epidemiological parameters including vector abundance, presence of alternative hosts in the field, seasonal movement between the hosts of concern and alternative or reservoir hosts, and weathers as well as climatic factors are important determinants epidemic development (Cunniffe et al. 2015; Jeger et al. 2004).

Wheat streak mosaic is an important vector borne viral disease of wheat (*Triticum aestivum* L) caused by *Wheat streak mosaic virus* (WSMV; Family: *Potyviridae*, Genus: *Tritimovirus*). The disease has been reported across the globe from the Middle East to Asia, Oceania, and South and North America (French and Stenger 2003; Navia et al. 2013). In the USA, wheat streak mosaic was first observed in 1919 near St. Louis, MO as “rosette disease.” It was later reported as “yellow mosaic” in Nebraska, USA in 1922

(McKinney 1937). It is now the most common viral pathogen of wheat in the Great Plains of North America (Burrows et al. 2015).

Wheat infected with WSMV can result in reduction in root mass and yield losses, depending upon agronomic and field conditions (Price et al. 2010; Rahman et al. 1974). The WSMV-infected plants typically show symptoms including stunting and yellow streaked leaves in mosaic patterns (McMullen and Waldstein 2010). In the Great Plains, yield losses range from 13 to 70 percent due to WSMV infection in mechanically inoculated trials (Rahman et al. 1974; Sharp et al. 2002; Miller et al. 2014). However, a yield loss caused by naturally infected WSMV in Kansas is estimated at an average of 1 to 2 percent annually (Appel et al. 2015). Yield reduction is greatest when the infection occurs in early stages of the plant's development (Hunger et al. 1992). In addition to crop stage, severity of symptoms and yield reductions can be affected by cultivar susceptibility, soil water availability, amount and types of fertilizers, and other environmental conditions (Miller et al. 2014; Lehnhoff et al. 2015; Velandia et al. 2010).

As an obligate pathogen, WSMV requires a vector, the wheat curl mite (WCM) *Aceria tosichella* Keifer (Slykhuis 1955), and alternative hosts for its survival and dispersal. WSMV is transmitted by nymphal and adult stages of WCM (Siriwetwivat 2006; Slykhuis 1955). WCMs can survive on more than 80 grass species including grassy weeds and crops (Navia et al. 2013; Somsen and Sill 1970). These alternative hosts serve as "green bridge," or green plant materials present between harvesting of one crop and planting of the next.

As a winter annual species, downy brome (*Bromus tectorum* L.), mostly germinates in the fall and matures in late spring (mid-July to early August), but it can

also behave as a spring annual species that germinates in the spring if fall moisture is inadequate (Harris 1967). If it germinates in the late spring it remains green throughout the fall (Ranabhat's personal observation for three years study period, 2013 - 2015) and may become a potential reservoir of WCM and WSMV. Likewise, volunteer wheat germinated just before, during, or immediately after harvest of wheat may act as a major source of WSMV inoculum for next season winter wheat (Wegulo et al. 2008). Green foxtail [*Setaria viridis* (L.) Beauv.] and corn (*Zea mays* L.) have also been identified as important green bridge species of WSMV in the Great Plain region (Ito et al. 2012; Somen and Sill 1970).

It has long been known that eliminating the green bridge and altering planting date reduce the risk of WSMV transmission (Hunger 2010; Hunger et al. 1992). However, increasing farm size hinders timely application of weed management practices to reduce the spread and abundance of alternative hosts. Also, the pressure to plant winter wheat early to increase yields in northern states is another barrier to WSMV management. Understanding the peak period of WCM movement and WSMV transmission from alternative hosts to the newly emerged wheat will provide a better management of disease in an area.

Although previous greenhouse and field studies have assessed the susceptibility of grass species to WSMV and WCM (Connin 1956; Ito et al. 2012; Somen and Sill 1970), there is little information available about the factors that determine variation in seasonal risk of virus and vector spread. Increased knowledge about the risk factors influencing vector abundance and WSMV infection status will contribute to our understanding of, and ability to predict, epidemics (Cunniffe et al. 2015; Jiang et al. 2005). The main

objective of this study was to assess the role of alternative hosts on risk of WCM infestation and WSMV infection of wheat in field conditions. Specifically, we identified the peak period of WCM infestation and WSMV infection during the fall. Additionally, we assessed the influence of alternative hosts on WCM infestation and WSMV infection to wheat.

Materials and Methods

Site Description

This experiment was conducted in 2013 and 2014 at the Arthur H. Post Agronomy Research Farm, Bozeman, MT, USA (45° 40' 29" N, 111° 09' 14" W, 1423 m elevation). The soil type at the site was Amsterdam-quagle silt loam (Soil Survey Staff NRCS, USDA, 2015). The mean temperature during our sampling months of August, September and October was 20°C, 15°C and 5°C respectively in 2013 and 18°C, 13°C and 10°C respectively in 2014 (USBR, Great Plains, Agrimet 2015).

Experimental Design

Each year, treatments were arranged in a randomized complete block design with six blocks. Each block consisted of six, 2 m × 2 m plots where alternative hosts of the WCM and WSMV were assigned randomly within blocks. Alternative hosts included 1) spring emerged downy brome; 2) fall emerged downy brome; 3) wheat planted to simulate pre-harvest volunteer; 4) wheat planted directly after harvest to simulate post-

harvest volunteer 5) corn, and 6) green foxtail. In 2013, bare ground control plots were placed in the center of four cardinal directions around the perimeter of the experimental blocks. The experiment was slightly modified in 2014, with the bare ground control treatment assigned randomly within each block.

To monitor WCM infestation and WSMV infection into the experimental area and within treatment plots, we used winter wheat (cv. Neeley) trap plants placed in the center of each plot. Trap plants were grown in cone-tainers (3.8 cm diameter, 21 cm depth, Ray Leach, model SC10, Stuewe & Sons, OR, USA) to Feekes growth stage 3 (three leaves) in greenhouse conditions at 21.1°C day and night with a 16:8 h, light:dark regimen. The planting media was a 1:1:1 mixture by volume of aerated stem pasteurized loam soil, washed concrete, Canadian Sphagnum peat moss mixed with AquaGro 2000G wetting agent, and Sunshine Mix number 1 (Sun Gro Horticulture Inc. Bellevue, WA). Four cone-tainers, each containing a trap plant were inserted into four 3.8 cm-diameter holes drilled in a 15.2 cm² x 0.31 cm thick piece of rigid plastic placed in a 30 cm² x 0.63 cm plywood with a 15 cm- diameter hole, all these structure were placed into a 17.8 cm diameter by 18 cm deep plastic bucket filled with water (Supplemental Fig. 2.5). The bucket was inserted into a hole excavated in the soil such that the top of the bucket was flush with the soil surface. Plants were covered with a screen made from hardware cloth with 1.3 × 1.3 cm openings to protect them from grazing animals and hail.

Alternative hosts were seeded by hand. Spring downy brome was seeded on June 5, 2013 and June 4, 2014 at rates of 1,580 seeds m⁻² by scattering the seeds in the plots and slightly incorporating them into the soil via raking. Fall downy brome plots were

established from naturally emerging downy brome in fall. Pre-harvest volunteer wheat plots were planted by hand-scattering seeds on plots at a rate of 100 seeds m⁻² on July 12 2013 and July 14 2014. Post-harvest volunteer wheat plots were similarly planted on August 1 2013 and July 30 2014. Sweet corn (*Z. mays* var. *rugose*, Early Sunglow hybrid 4.50) was planted on June 5 2013 with 10 cm seed spacing and approximately 3 cm depth at 80 seeds m⁻². Due to low emergence and difficulty growing sweet corn in dryland conditions, field corn (*Z. mays*, P8107HR, Pioneer) was hand seeded on June 4 2014 with 10 cm seed spacing at a 4 cm depth at 80 seeds m⁻². Green foxtail was seeded during the first week on June 5, 2013 and June 4, 2014 at rates of 9,395 seeds m⁻², by scattering the seeds in the plots and incorporating them into the soil by raking.

Plot Sampling and Greenhouse Protocols

To measure WCM infestation and WSMV infection, trap wheat plants were placed in each plot and removed after seven days from August 2 to October 25, 2013 and from August 5 to October 28, 2014. This period was selected to span the range of agronomic events potentially important to the WCM-WSMV-host complex. In Montana, typical winter and spring wheat harvest dates range from August to mid-September whereas, and typical winter wheat planting is from mid-September to mid-October. About 60 -70 % of winter wheat emerges in third week of October (NASS, 2014). After collecting trap wheat plants from the field, they were kept in the greenhouse to allow WCM populations and WSMV infection to develop. To prevent WCM movement, individual wheat plants were covered with plastic cages (Visipak, 3.8 cm diameter × 61

cm height clear plastic tubes) with three vent holes (2.5×5 cm) cut in each tube and covered with a fine nylon lab pak mesh (25 μ m; Sefer AG, Switzerland). All plants were kept for two weeks in the greenhouse at 21.1°C day and night with a 16:8 h, light: dark regimen. At the end of this period, trap plants were harvested and WCM presence or absence was recorded on each plant. One youngest, fully expanded leaf on each plant was kept in a re-closable plastic bag for WSMV detection.

Sampling of Alternative Host for Direct

Monitoring of Mite and Virus on Host Plants: Six alternative host plants from

each treatment except fall downy brome were randomly sampled in the field to assess the WCM number and WSMV infection on the alternative host plants. Fall downy brome was not sampled because it had already senescence in both years. In 2013, host plants were sampled once at mid-September to know the WCM abundance and WSMV infection. However, in 2014, the sampling method was modified as host plants were sampled three times: at 1399, 1956 and 2373 GDD. The number of mites per plant was counted and the youngest, fully expanded leaf per plant was kept in a re-closable plastic bag to detect WSMV infection.

Detection of WSMV

WSMV infection was evaluated by using an indirect enzyme-linked immunosorbent assay (ELISA) as described by Ito et al. (2012) with leaves processed individually. Six to eight negative control samples were systematically distributed in the wells of a plate to reduce bias in values of optical density caused by position of samples

in the plate. The mean and standard deviation of the negative control of healthy wheat samples on each plate were calculated and used to set a probabilistic optical density threshold at three standard deviations above the mean. Samples above this threshold were considered infected with WSMV (Miller et al. 2014).

Growing Degree Day Calculation

Meteorological data were obtained from the United States Bureau of Reclamation, Great Plains, Agrimet station (www.usbr.gov/gp/agrimet) using the BOZM weather station. The daily temperature data during the experimental period of August to October of both years were used to calculate growing degree days Growing degree days of spring wheat (GDD hereafter), calculated as:

$$\text{GDD} = \sum [(T_{\max} + T_{\min})/2] - T_{\text{base}} \quad (\text{eq. 1})$$

where, T_{\max} and T_{\min} are daily maximum and minimum temperature respectively and T_{base} is the base temperature. A T_{base} 0°C was used for wheat (McMaster and Wilhelm 1997). If the daily mean temperature was less than the base, it was set equal to the base temperature during calculation of GDD. The planting date of spring wheat at the study site was used to set the starting point for GDD calculation and GDDs were used as a predictor variable of WCM and WSMV movement.

Statistical Analysis of Monitoring Trap Plant

To assess patterns of WCM infestation and WSMV infection on trap plants in response to GDD and host species identity, we fitted separate generalized linear mixed

effect models (GLMMs) with binomial distribution for each response. All analyses were performed in R version 3.0.1 (R- Development Core Team, 2014) using the lme4 package (Bates et al. 2015). The response variable in each model was the WCM infestation and WSMV infection on trap plants out of the total number of plants sampled from alternative host plots. In the models, we used GDD, host species identity and year as fixed effects. The random effect of alternative hosts was nested within block. To assess the best predictor of the response of WCM infestation or WSMV infection, we compared nested models (see Supplemental Table 2.1 for a list of models). Small-sampled-sized corrected Akaike's Information Criterion (AICc) was used to select best-fit model, the model with lowest AICc values was selected for interpretation (Burnham and Anderson 2002; Garrett et al. 2004; Raffalovich et al. 2008; Bolker et al. 2009).

To assess the impact of alternative hosts in disease risk, we compared the rate of infection of trap plants between alternative hosts and bare ground control plots. GDD was used as the categorical exploratory variable to account for the variability of patterns of WCM infestation and WSMV infection with sampling dates. For the comparisons, total infection (probability = 1) and zero infection (probability = 0) were transformed as the logit function of binomial distribution is undefined at these values (Ramsey and Schafer 2002; Agresti and Coull 1998; Agresti and Caffo 2000; Warton and Hui 2011) (see supplementary table 1 for details). In all tables and figure, results are presented as the percentage of infected plants per plot without transformation. The best-fit model was selected using AICc. MULTCOMP package with general linear hypothesis test (glht) used to compare host species treatments with control. Contrasts were defined between

alternative host plants and control interaction within a particular sampling date (Hothorn et al. 2015).

Statistical Analysis of Direct Monitoring of Host Plant

To assess WCM abundance on alternative hosts, we fitted generalized linear mixed effect models (GLMMs) with Poisson distribution (Ramsey and Schafer 2002; Zuur et. al 2009). Wheat curl mite abundance was modeled with GDD and alternative hosts fitted as fixed effects and block as random effect factor. We utilized a similar statistical analysis for model selection procedure and multiple comparisons as described above.

Results

Influence of Alternative Host Species and GDD on WCM Infestation of Wheat

The relationship between GDD and probability of WCM infestation on trap plants was non-linear and best described by a third order polynomial function of GDD (Supplemental table 2.1). This relationship was inconsistent between years and across alternative hosts ($\text{GDD}^3 \times \text{year} \times \text{alternative hosts}$, $\chi^2 = 55$, $\text{df} = 18$, $P < 0.01$). Probability of WCM infestation on trap plants varied by alternative hosts ($\chi^2 = 50.3$, $\text{df} = 6$, $P < 0.01$) and GDD ($\chi^2 = 285.3$, $\text{df} = 3$, $P < 0.01$).

The impact of alternative hosts on WCM infestation varied between years. In 2013, alternative hosts had little effect on risk of WCM infestation. In control plots, the probability of trap plants becoming infested with WCM peaked between 1800 to 2300

GDD (Fig. 2.1) with maximum of 81% infestation (Supplementary Table 2.2). The probability of infestation on trap plants from all alternative host species was similar to the probability of trap plants infested in the bare ground control ($P > 0.05$) except spring downy brome at 1704 GDD and fall downy brome at 2276 GDD that had either higher or lower WCM infestation than control plots, respectively ($P < 0.05$) (Fig 2.1 and Supplementary Table 2.2). In 2014, alternative hosts modified the risk of mite infestation at the beginning of fall and the peak period of WCM infestation on alternative host plots varied across host species and GDD (Fig. 2.1). The probability of trap plants infested with WCM peaked between 1500 to 1800 GDD with maximum of 42% infestation in control plots (Fig 2.1 and Supplementary Table 2.3). The likelihood of infestation on trap plants was higher in spring and fall downy brome as well as pre-harvest volunteer wheat plots than control plots during most of the fall ($P < 0.01$; Supplementary Table 2.3). The probability of WCM infestation of corn and post-harvest volunteer wheat plots was similar to control plots during the fall ($P > 0.05$).

Influence of Alternative Host Species and GDD on WSMV Infection to Wheat

The WSMV infection on alternative hosts differed by host species and GDD. There was a non-linear relationship between GDD and probability of WSMV infection on trap plants and the rate of infection was best described by the third order polynomial function (Supplemental Table 2.1). The relationship between GDD and probability of WSMV infection on trap plants was inconsistent between years across alternative hosts ($\text{GDD}^3 \times \text{year} \times \text{alternative hosts}$, $\chi^2 = 41$, $\text{df} = 18$, $P < 0.01$). The probability of WSMV

infection of trap plants varied by alternative hosts ($\chi^2 = 129.8$, $df = 6$, $P < 0.01$) and GDD³ ($\chi^2 = 106.4$, $df = 3$, $P < 0.01$).

Alternative hosts influenced the probability of WSMV infection. In 2013, the peak period of WSMV infection of trap plants in control plots occurred at 1566 GDD and in the alternative host plots between 1700 to 1900 GDD (Fig. 2.2 and Supplemental Table 2.4). The probability of WSMV infection of trap plants in spring seeded downy brome at 1849 GDD ($P = 0.01$), pre-harvest volunteer wheat and corn at 1704 GDD ($P = 0.01$), green foxtail at 2276 GDD ($P = 0.01$), and post-harvest volunteer wheat at 1849 and 2276 GDD ($P < 0.01$) was higher than in control plots. Results of WSMV infection of trap plants in fall downy brome plots was influenced by herbivory, as trap plants were consumed by insect and small mammal herbivores up to the mid fall. Therefore, the risk of WSMV infection in fall downy brome plots did not accurately measure since many trap plants were not exposed to vector as they were eaten by herbivores. In 2014, alternative hosts influenced the probability of virus infection of trap plants during the mid and late fall. There was a greater risk of WSMV infection in 2014, though there was a lower risk of WCM infestation when compared to 2013 ($P < 0.01$). The probability of trap plants infected with WSMV in control plots peaked at 1399 and 2294 GDD (Fig. 2.2) with maximum of 83% of trap plants infected (Supplemental Table 2.5). Relative to controls, mean probabilities of WSMV infection of trap plants were higher in the spring downy brome plots at 2329 and 2373 GDDs ($P < 0.01$) and pre-harvest volunteer wheat plots at 1867 to 1956 GDDs ($P < 0.01$, Supplemental Table 2.5). The probability of WSMV infection of trap plants in corn and post-harvest volunteer wheat plots was higher than the control at 1672 GDD and 1956 GDD, respectively ($P = 0.01$). Green foxtail plots

had higher percentages of WSMV infection of trap plants than control plots at 1867 and 2329 GDD ($P < 0.01$). Post-harvest volunteer wheat and green foxtail had lower WSMV infection on trap plants than the control at most of the other GDD ($P < 0.01$).

Direct Monitoring of Alternative Host

Alternative hosts were directly monitored once in 2013 and three times in 2014. In 2013, spring downy brome had high WCM abundance (107 ± 16 , mean and SE), and corn and green foxtail had negligible WCM abundance (below 1 in average). In 2014, WCM abundance varied among host species ($\chi^2 = 6.1$, $df = 2$, $P = 0.04$) and GDD ($\chi^2 = 394.7$, $df = 2$, $P < 0.01$) (Fig. 2.3). At 1399 GDD, no WCM were recorded in pre-harvest and post-harvest volunteer wheat. Spring downy brome had higher WCM's abundance of 169 ± 86 (mean + SE) at 1956 GDD then pre-harvest and post-harvest volunteer wheat ($P < 0.01$). These host species had similar WCM abundance at 2373 GDD ($P > 0.05$). No WCMs were found on corn and green foxtail.

WSMV infection in alternate hosts was not measured in 2013, but varied among hosts ($\chi^2 = 202.6$, $df = 3$, $P < 0.01$) and GDD ($\chi^2 = 143.1$, $df = 2$, $P < 0.01$) in 2014. At 1399 GDD, no WSMV was recorded in pre-harvest and post-harvest volunteer wheat whereas 72% of spring downy brome plants were infected by WSMV (Fig. 2.4). Seventeen percent of corn plants were infected at 1399 GDD but there was no infection later. Spring downy brome and both simulated volunteer wheat treatments had WSMV infection at 1956 and 2373 GDD, and the infection levels did not differ from one another ($P > 0.05$) (Fig. 2.4). There was 0% infection of green foxtail by WSMV.

Discussion

A better understanding of WCM dispersal onto potential “green bridge” alternative hosts is crucial for successful management of wheat streak mosaic. Furthermore, the information about WCM and WSMV persistence on the alternative hosts, and host role in disease spread into wheat fields will facilitate the development of effective ecologically-based management strategies. In this study, I assessed temporal dynamics of WCM and WSMV during the fall, the impact of alternative hosts on disease risk, and the ability to predict disease risk using GDD.

The initial peak of WCM infestation and WSMV infection at the beginning of the fall in control plots coincided with the timing of maturation of several grasses and harvest of cereal crops. In accordance with previous studies, viruliferous mites moved from surrounding cereal fields before and during harvest (Nault and Styer 1969; Thomas et al. 2004). In Montana, the typical period of winter and spring wheat harvesting is from August to mid-September (NASS 2014). Wegulo et al. (2008) also described that a “mite rain” is primarily triggered by the senescence and harvesting of wheat. The time of harvesting and presence of green plants increases the survival and reproduction of WCM and WSMV in the field after harvesting of cereal crops (Somsen and Sill 1970). Mites then survive and reproduce on alternative hosts that acted as green bridge hosts for mite and virus.

Risk of WCM infestation and WSMV infection on trap plants was increased from some alternative hosts. Corn, green foxtail, and post-harvest volunteer wheat had similar WCM infestation compared to controls in both years indicating that these hosts do not

increase the disease risk, at least for the studied region. Interestingly, a decrease in WSMV infection on trap plants was also recorded from these hosts plots compared to control plots at beginning and end of the fall, even though WCM infestation rate was high. The reason of low WSMV infection is out of the scope of this study and further research is needed to test relation of virus titer in WCM and the rate of WSMV transmission from alternative hosts to wheat. In contrast, downy brome and pre-harvest volunteer wheat can increase disease risk as rate of WCM infestation and WSMV infection was increased especially at the late fall (after 2128 GDD or third week of September) compared to control in 2014. Low WCM infestation and WSMV infection in the late fall (after mid-September) in 2013 and high infestation and infection during that period in 2014 corresponds with low daily temperature and relatively high temperature in 2013 and 2014 respectively. The mean minimum and maximum daily temperature recorded from mid-September was 0.8° C and 12.9° C in 2013; 4.4° C and 18.6° C in 2014 (USBR, Great Plains, Agrimet 2015). Warm temperatures favor dispersal of mites (Slykhuis et al. 1957). These hosts play important role to increase the disease risk in relatively high temperature. The higher rate of WCM infestation during late fall at the time of emergence of winter wheat increased the likelihood of infestation as about 60 -70 % of winter wheat emerged up to third weeks of October (NASS, 2014).

Downy brome emerged in the spring and volunteer wheat was associated with the increase in disease risk for winter wheat measured by direct sampling of host plants. The result of high WCM abundance and WSMV infection on spring downy brome and both simulated volunteer wheat plants indicates that WCM can survive and reproduced

successfully as I observed different growth stages of mites on them. These hosts can serve as WSMV inoculum sources for the transmission by WCM to winter wheat, perpetuating the disease cycle of several plant viruses and their vectors (Bowden et al. 1991, Seifers et al. 2002, 2010). No WCMs were observed from corn leaves, but the plants were infected WSMV. This may be due to the difficulties of finding mites on corn leaves (Somen and Sill 1970). Contrary to previous results (Ito et al. 2012; Somen and Sill 1970), in this study there were no WCM or WSMV on green foxtail. Research is needed to verify the variation of green foxtail susceptibility with multiple isolates of virus and mite biotypes.

The temporal dynamics of mite and virus and the ability of alternative hosts to support vector population and persistence of virus throughout the fall fallow periods presented in this study provide essential information to make appropriate disease management decisions. The information about length of peak period of mite infestation and virus infection may help growers to adjust crop planting date, using tolerant or resistant winter wheat varieties, increase crop rotation, and apply appropriate weed management strategy based on the alternative hosts present in the field.

Winter wheat emerged even at the week of moderate WSMV infection that will experience cumulative high risk over the rest of the fall due to longer exposure. For example, if we have moderate probability of infection, 40 % and 60 % in first and second week respectively, the cumulative probability of WSMV infection on third week will be 76 %. Therefore, for disease management, risk of disease will be low when winter wheat emerged at the period of continuous low WSMV infection with short period of exposure.

These agronomic practices help to break disease cycle by escaping peak period of potential exposure to viruliferous mite (Holtzer et al. 1996).

WCM infestation and WSMV infection in winter wheat in the fall was not predicted by the extended GDD of spring wheat of that growing season in the fall. Movement of WCM is influenced by year to year variability in weather conditions and agronomic practices like crop phenology and harvest (Lanning et al. 2010; Thomas et al. 2004). Continuous low temperature reduces the risk of WSMV infection. Daily mean temperature or minimum and maximum temperature may provide possible prediction the risk of mite infestation and virus infection. However, the results of risk of WCM infestation and WMSV infection and disease dynamics during fall, help to make decision on planting date of winter wheat. Additionally, the ability of alternative hosts to support vector population and virus could help growers to identify the high-risk alternative hosts of WCM and target these species for control. The information obtained in this research could also be used to develop a forecasting of WSMV epidemic risk under changing climatic scenarios. Risk assessment forecasting models assist growers in planning more profitable cropping option to reduce the potential for crop failure.

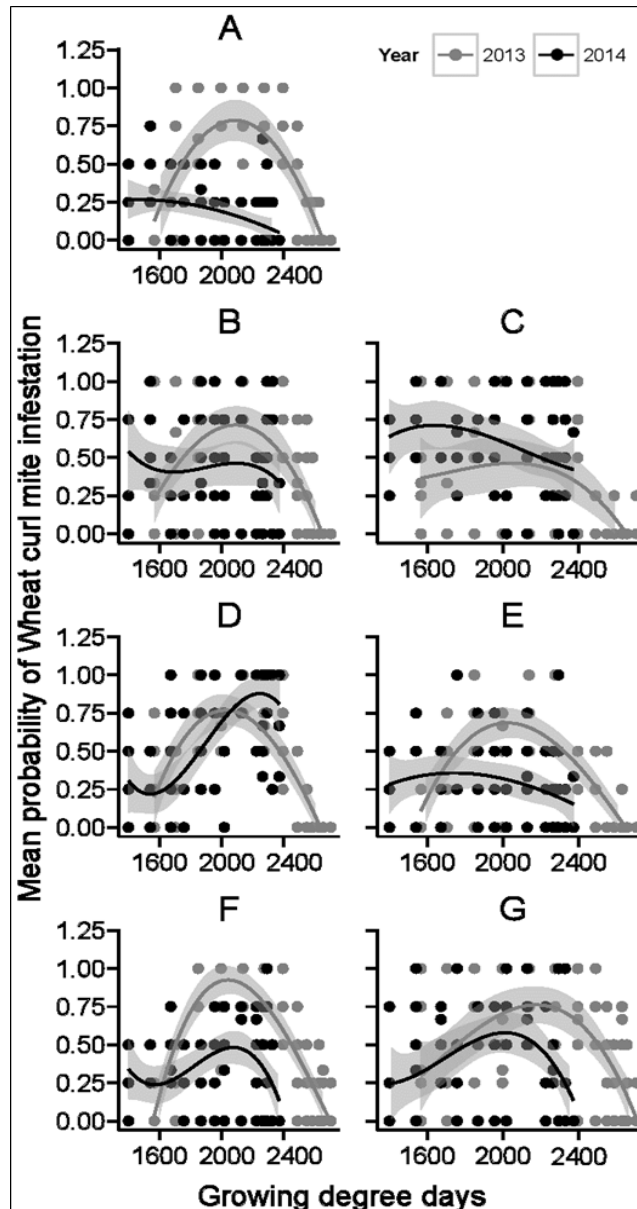


Fig. 2.1. Probability of wheat curl mite (WCM, *Aceria tosichella*) infestation of wheat trap plants from alternative host plots in 2013 and 2014. Data is expressed as the mean probability of WCM infestation measured in one week increments. The date of trap plant collection was transformed to the accumulated growing degree days for spring wheat in order to compare data between years. Alternative host plots include: **A**, bare ground control; **B**, spring downy brome; **C**, fall downy brome; **D**, pre-harvest volunteer wheat; **E**, post-harvest volunteer wheat; **F**, corn; and **G**, green foxtail. Each data point represents an individual plot in a particular sampling week. The solid line is the fitted line from generalized mixed effect models with binomial distribution and the shaded area is the 95% confidence interval.

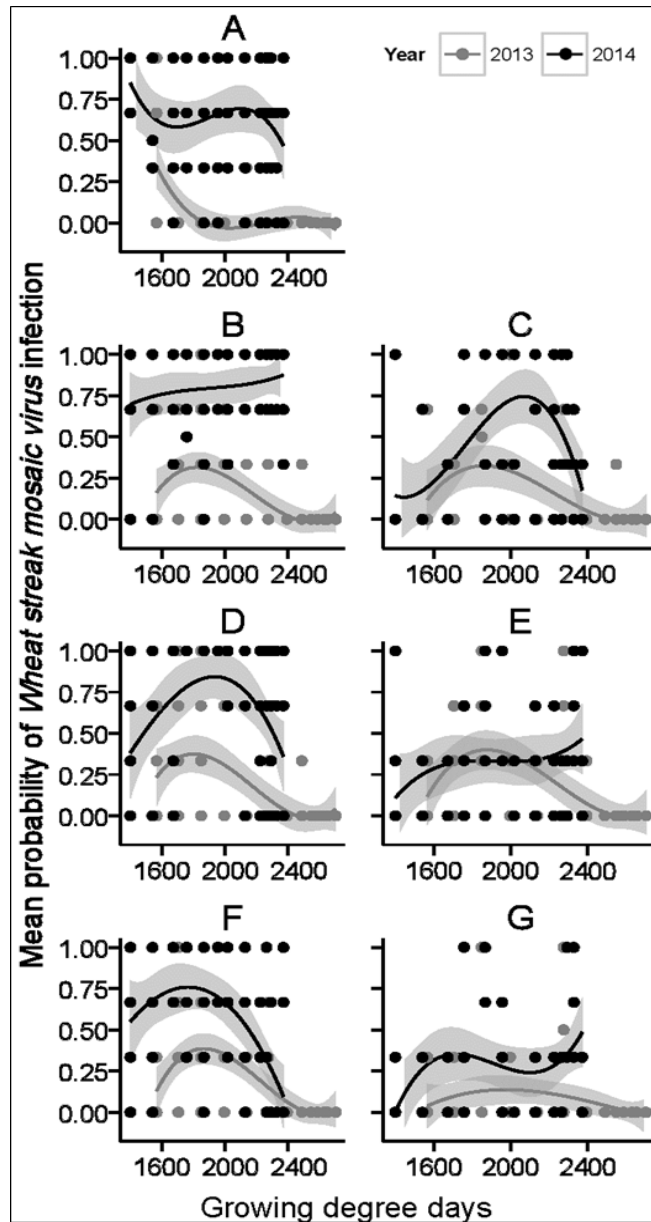


Fig. 2.2. Probability of *Wheat streak mosaic virus* (WSMV) infection of wheat trap plants from alternative host plots in 2013 and 2014. Data is expressed as the mean probability of WSMV infection measured in one week increments. The date of trap plant collection was transformed to the accumulated growing degree days for spring wheat in order to compare data between years. Alternative host plots include: **A**, bare ground control; **B**, spring downy brome; **C**, fall downy brome; **D**, pre-harvest volunteer wheat; **E**, post-harvest volunteer wheat; **F**, corn; and **G**, green foxtail. Each data point represents an individual plot in a particular sampling week. The solid line is the fitted line from generalized mixed effect models with binomial distribution and the shaded area is the 95% confidence interval.

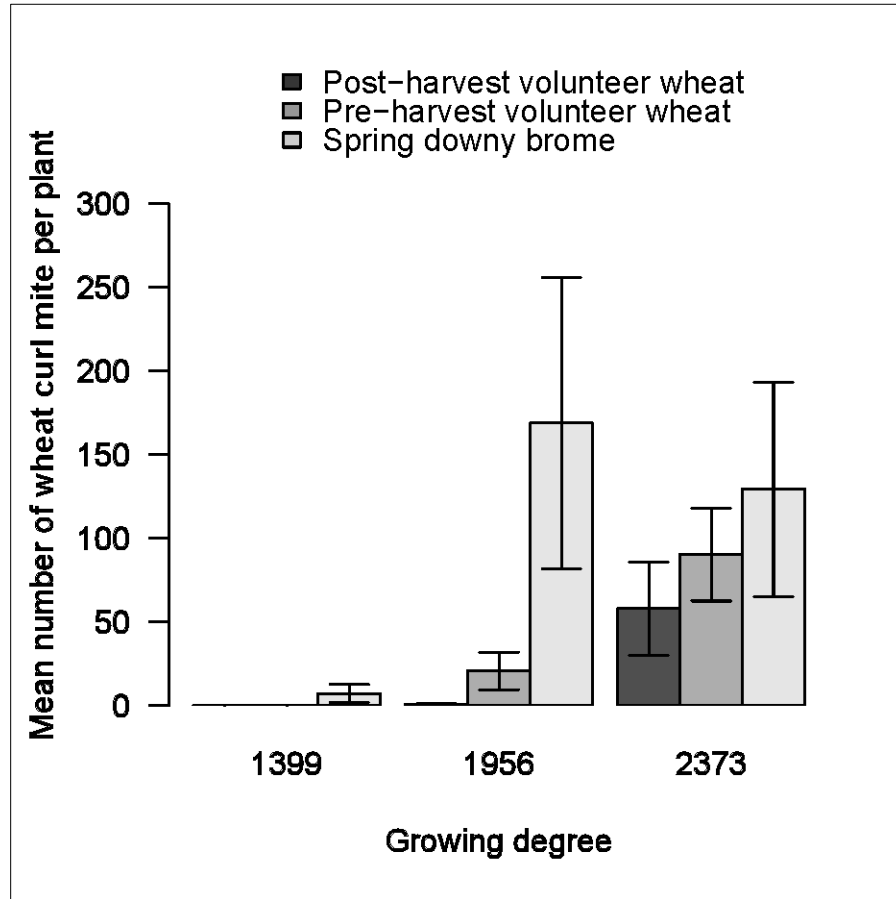


Fig. 2.3. Mean and standard error of wheat curl mites (WCM, *Aceria tosichella*) per plant on alternative hosts in 2014. The date of host plant collection was transformed to the accumulated growing degree days (GDD) for spring wheat. Total number of observations per GDD and host combination = 36, six plants per replication with six replications.

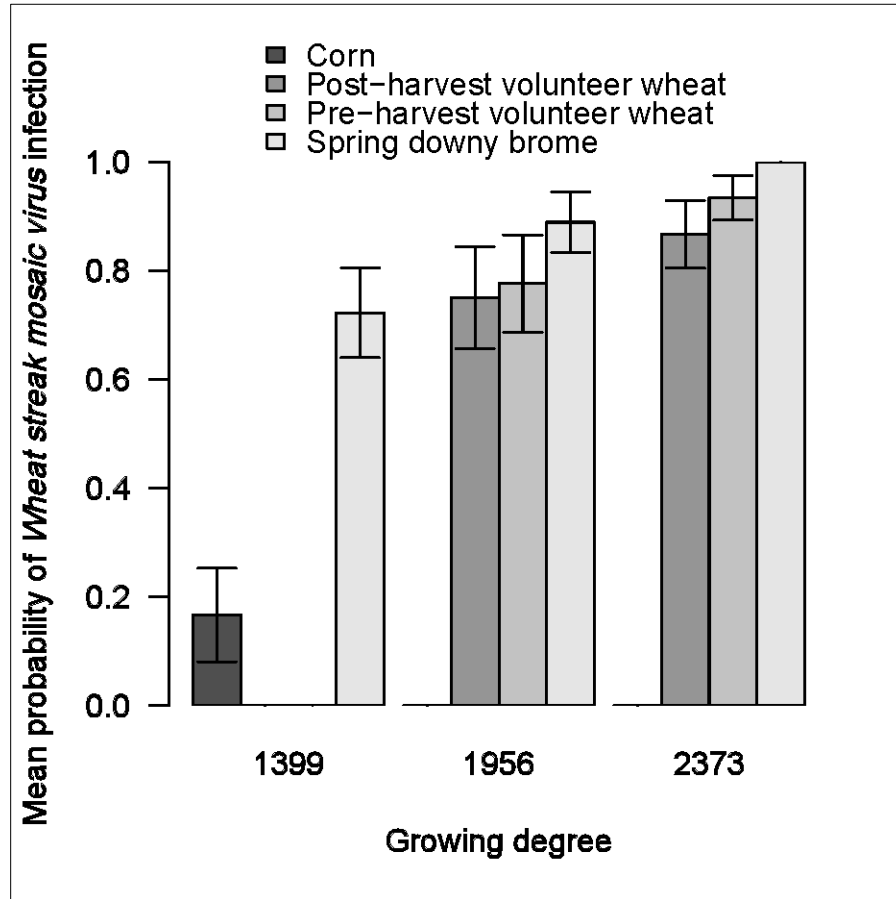


Fig. 2.4. Mean probability of *Wheat streak mosaic virus* infection and standard error per plant on alternative hosts in 2014. The date of host plant collection was transformed to the accumulated growing degree days (GDD) for spring wheat. Total number of observations per GDD and host combination = 36, six plants per replication with six replications.

Supplemented Table 2.1. Small-sample-size corrected version of Akaike's information criterion (AICc) scores and associated *P* value from generalized linear mixed effect models with binomial distribution. Treatment nested with block was fitted as random effect and the probability of wheat curl mite (WCM, *Aceria tosichella* Keifer) infestation and *Wheat streak mosaic virus* (WSMV) infection as response variable (total number of observations =1066), growing degree days (GDD), year and Alternative hosts are predictor variables, where *k* is the number of parameters in the models

Variables	<i>k</i>	WCM ^z		WSMV ^z	
		AICc	<i>P</i> value	AICc	<i>P</i> value
Intercept only	3	3420	-	3118	-
GDD	4	3323	<0.0001	2897	<0.0001
Year	4	3405	1	2437	<0.0001
(GDD) ²	5	2967	<0.0001	2604	1
(GDD) ³	6	2898	<0.0001	2526	<0.0001
Alternative hosts	9	3396	1	3092	1
GDD + Year + Alternative hosts	11	3300	<0.0001	2361	<0.0001
(GDD) ² + Year + Alternative hosts	12	2922	<0.0001	2200	<0.0001
(GDD) ³ + Year + Alternative hosts	13	2820	<0.0001	2193	<0.0001
GDD * Year * Alternative hosts	30	3094	1	2198	0.02
(GDD) ² * Year * Alternative hosts	44	2630	<0.0001	2071	<0.0001
(GDD) ³ * Year * Alternative hosts	58	2606	<0.0001	2064	<0.0001

^z Bold values indicates the best-fitted model with lowest AICc score

Supplemented Table 2.2. Mean percentage of wheat curl mite (*Aceria tosichella* Keifer) infested wheat trap plants in alternative host plots in 2013^x

Alternative host treatment ^y	August			September				October					
	Growing degree days of trap plants sampling ^z												
	1566	1704	1849	1997	2138	2276	2396	2490	2550	2588	2630	2656	2706
Bare ground control	8	44	79	63	81	67	81	31	6	6	13	0	0
Spring downy brome	17	83 A	92	88	96	94	88	40	13	0	8	0	0
Fall downy brome	42	33	43	25	70	25 a	67	8	0	4	0	0	4
Pre-harvest volunteer wheat	33	21	88	61	83	74	71	8	4	0	0	0	0
Post-harvest volunteer wheat	17	33	63	56	79	67	46	17	8	0	4	0	0
Corn	13	29	96	81	96	79	75	42	17	4	25	10	8
Green foxtail	39	21	79	55	88	75	61	58	38	13	43	13	4

^z Upper case letter within column indicate significant ($P < 0.05$) higher and lower case letter within column indicates significant ($P < 0.05$) lower mean percentage of wheat curl mite infested wheat trap plants in alternative host plots than control plots according to multiple comparison procedure , general linear hypothesis test (ghlt)

^y Total number of observations per GDD and host combination = 24, four trap plants per replication, six replications.

^x GDD was used as the categorical exploratory variable to account for the variability of patterns of WCM infestation and WSMV infection with sampling dates. For the comparisons, data of total infection (probability = 1) and zero infection (probability = 0) were transformed as the logit function of binomial distribution is undefined at these values (Ramsey and Schafer 2002) During transformation, because of small sample size, sample size of data that had probability 1 and 0 transformed into five times of its original sample size and added one success and failure (Agresti and Caffo 2000; Agresti and Coul 1998; Warton and Hui 2011). This transformation changes the probabilities zero to close to zero and one to close to one.

Supplemented Table 2.3. Mean percentage of wheat curl mite (*Aceria tosichella* Keifer) infested wheat trap plants in alternative host plots in 2014

Alternative host treatment ^y	August				September				October				
	Growing degree days of trap plants sampling ^z												
	1399	1540	1672	1757	1867	1956	2018	2128	2226	2265	2294	2329	2373
Bare ground control	17	42	25	29	22	13	13	8	13	19	17	4	0
Spring downy brome	63 A	88	74 A	50	85 A	67A	75 A	88 A	83 A	86 A	96 A	42	22
Fall downy brome	54	88 A	80 A	53	67	67 A	42	46	58 A	67 A	50	55 A	13
Pre-harvest volunteer wheat	29	21	46	17	54	71 A	58	92 A	92 A	75 A	96 A	88 A	75 A
Post-harvest volunteer wheat	21	50	29	38	29	25	33	35	13	25	42	13	6
Corn	25	38	43	17	29	38	43	60	40	38	50	17	0
Green foxtail	17	43	53	35	38	58	54	68 A	38	35	50	25	0

^z Upper case letter within column indicate significant ($P < 0.05$) higher and lower case letter within column indicates significant ($P < 0.05$) lower mean percentage of wheat curl mite infested wheat trap plants in alternative host plots than control plots according to multiple comparison procedure , general linear hypothesis test (ghlt)

^y Total number of observations per Growing degree days and host combination = 24, four trap plants per replication, six replications.

Supplemented Table 2.4. Mean percentage of *Wheat streak mosaic virus* infested wheat trap plants in alternative host plots in 2013

Alternative host treatment ^y	August				September				October				
	Growing degree days of trap plants sampling ^z												
	1566	1704	1849	1997	2138	2276	2396	2490	2550	2588	2630	2656	2706
Bare ground control	42	0	0	0	8	0	0	0	0	0	0	0	0
Spring downy brome	11	33	44 A	17	6	17	0	6	0	0	0	0	0
Fall downy brome	17	8	61 ^x	33 ^x	0	67 ^w	0	0	6	0	0	0	0
Pre-harvest volunteer wheat	17 a	44 A	44	28	0	22	0	6	0	0	0	0	0
Post-harvest volunteer wheat	6 a	33	80 A	6	6	53 A	6	0	0	0	0	0	0
Corn	6 a	44 A	44	22	11	39	0	0	0	0	0	0	0
Green foxtail	6 a	6	17	13	0	37 A	0	0	0	0	0	0	0

^z Upper case letter within column indicate significant ($P < 0.05$) higher and lower case letter within column indicates significant ($P < 0.05$) lower mean percentage of wheat curl mite infested wheat trap plants in alternative host plots than control plots according to multiple comparison procedure, general linear hypothesis test (ghlt).

^y Total number of observations per GDD and host combination = 24, four trap plants per replication, six replications.

^x Total number of observation = 8 due to herbivory.

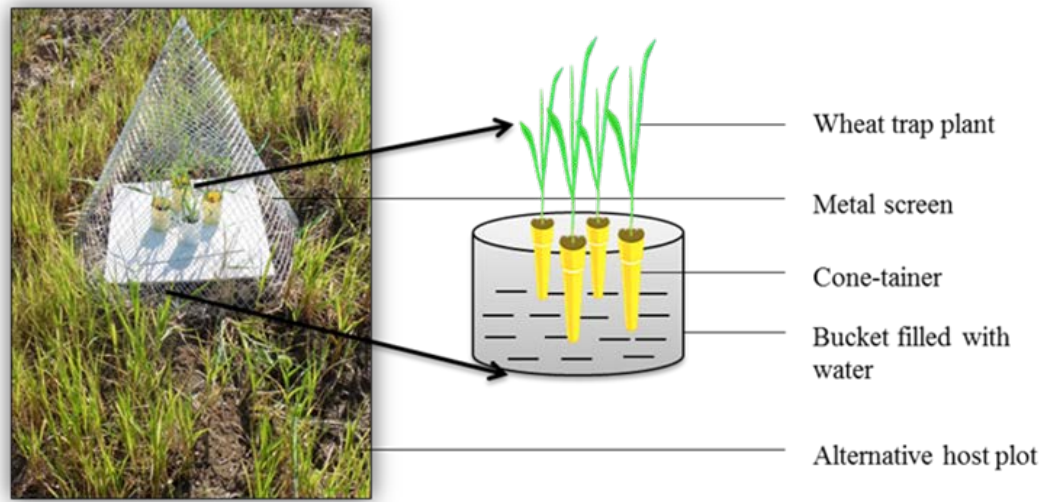
^w Total number of observation = 3 due to herbivory

Supplemented Table 2.5. Mean percentage of *Wheat streak mosaic virus* infested wheat trap plants in alternative host plots in 2014

Alternative host treatment ^y	August				September				October				
	Growing degree days of trap plants sampling ^z												
	1399	1540	1672	1757	1867	1956	2018	2128	2226	2265	2294	2329	2373
Bare ground control	83	69	44	78	56	61	72	61	67	61	83	39	44
Spring downy brome	67	78	83	81	61	89	78	78	94	83	89	83 A	83 A
Fall downy brome	17 a	11 a	11 a	94	44	55	72	61	67	56	78	22	6
Pre-harvest volunteer wheat	56	28	50	100	100 A	100 A	89	61	39	50	44	67	44
Post-harvest volunteer wheat	22 a	17 a	6 a	22 a	56	89 A	11 a	33	28	11 a	11 a	61	72
Corn	61	50	89 A	78	61	83	61	56	39	44	11 a	0	29
Green foxtail	11 a	6 a	17 a	50	83 A	17	0 a	11 a	11 a	11 a	78	83 A	11

^z Upper case letter within column indicate significant ($P < 0.05$) higher and lower case letter within column indicates significant ($P < 0.05$) lower mean percentage of wheat curl mite infested wheat trap plants in alternative host plots than control plots according to multiple comparison procedure , general linear hypothesis test (ghlt)

^y Total number of observations per GDD and host combination = 24, four trap plants per replication, six replications



Supplemental Fig. 2.5. Design for monitoring wheat curl mite (*Aceria tosichella* Keifer) infestation and *Wheat streak mosaic virus* (WSMV) on wheat trap plants grown in cone-tainers. The cone-tainer assembly was placed in a water-filled bucket flush with the soil surface to supply water for trap plants.

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CHAPTER THREE

IMPACT OF PLANTING DATE, NITROGEN APPLICATION TIMING, AND CROP
VARIETY ON WHEAT STREAK MOSAIC VIRUS INCIDENCE IN WINTER
WHEATIntroduction

Cultural practices are prophylactic and sustainable approaches for plant disease management (Noris and Caswell-Chen 2003). These practices work indirectly by altering hosts or environmental conditions in ways that inhibit crop disease incidence and spread (Ogle and Dale 1997; Hesler et al. 2005). Disease management is based on three principles: reducing disease inoculum, delaying the onset of disease to decrease the rate of disease progress, and minimizing the duration of the epidemics (Arneson 2011; Campbell and Madden 1990).

Wheat streak mosaic is a common viral disease of cereal crops caused by *Wheat streak mosaic virus* (WSMV). WSMV is transmitted by the wheat curl mite (*Aceria tosichella* Kiefer, WCM). This viral disease causes sporadic epidemics on wheat worldwide (Navia et al. 2013; Ellis et al. 2003). In the USA, wheat streak mosaic is the most common viral disease of wheat in the Great Plains (Burrows et al. 2015). The symptoms of WSMV include stunted growth, yellow streaked leaves with a mosaic pattern, reduced root mass, and yield and seed quality reduction (Rahman et al. 1974; Price et al. 2010). Yield loss caused by naturally infected WSMV in Kansas is estimated at an average of 1 to 2 % annually (Appel et al. 2015). However, the estimation of a 1%

yield loss turns into million dollars loss to the total wheat production (Burrows et al. 2015).

Management of the WSMV is complicated by its broad range of hosts that act as a “green bridge” from wheat harvest to the emergence of newly planted winter wheat (Lu et al. 2011; Navia et al. 2013; Zhang et al. 2015). Crops including barley (*Hordeum vulgare*), corn (*Zea mays*), foxtail millet (*Setaria italica*), pearl millet (*Pennisetum glaucum*), and cereal rye (*Secale cereal*); as well as volunteer wheat and grassy weeds act as alternative or reservoir hosts of WCM and WSMV and have a significant role in the survival and spread of WCM and the epidemiology of WSMV (Somsen and Sill 1970). Control of these alternative hosts to break the “green bridge” is an effective management tactic for WSMV. However, the efficacy of commonly used methods of “green bridge” management such as herbicide and tillage depend upon farm management and weather conditions. For example, synthetic herbicide applications are not an option in organic systems or may not be sustainable due to the presence of herbicide resistant weed biotypes and non-target effects of herbicide (Powles and Yu 2010). In addition, plants may take days to weeks to die following an herbicide application, allowing time for the WCM to move to susceptible host plants downwind of the inoculum source (Thomas et al. 2004; Jiang et al. 2005). Tillage is also used to control alternative hosts but it is not a viable option in no-till cropping systems and its excessive use could result in soil erosion (Greer et al. 2006). Additionally, WCM can survive outside the host for up to a week depending upon temperature and relative humidity (Wosula et al. 2015).

The patho-system of WSMV is complex consisting of host, virus, vector, environment, and impact of agronomic practices on their interaction. Due to this

complexity, research focused on a static single disease management practice could not provide effective, long term solution for WSMV management. There is no effective chemical option available for the control of WSMV or WCM (Harvey et al. 1979; Murphy 2016) and preventive cultural management practices are the key for successful long term management. The integrations of multiple cultural management tactics including planting date, nitrogen (N) application timing, and crop variety represents a potential option for effective long-term management of the WSMV.

Planting date can influence WSMV incidence and severity as wheat crop susceptibility is affected by growth stage at the time of infection (Hunger et al. 1992). It has been observed that WSMV impact on wheat yield is high when infection occurred at or prior to tillering and relatively lower at later growth stages (Hunger et al. 1992; Sill and Agusiobo 1955). Early seedling of winter wheat increases the duration of crop exposure to WCM and WSMV as mite immigration peaks during the late stages of maturation and the harvesting period of cereal grain crops (results of Chapter 2; Wegulo et al. 2008).

Along with its impact on yield and grain protein content (Jones and Olson-Rutz 2012) N can influence disease incidence and severity by affecting biochemistry and host quality for vector and pathogen reproduction (Dordas 2008; Walters and Bingham 2007). Past studies have demonstrated that viral infection is affected by the nutrient type and quantity (Huber and Watson 1974; Borer et al. 2010) and the effect of nutrients differed even in closely related viral species (Lacroix et al. 2014). For example, *Barley yellow dwarf* (BYDV-PAV) had higher infection rates with high N and phosphorus (P) but infection rates of *Cereal yellow dwarf virus* (CYDV-RPV) decreased with P supply

(Lacroix et al. 2014). N concentrations may increase herbivore damage by increasing herbivore preference, performance, and reproduction (Awmack and Leather 2002; Throop and Lerdaun 2004). Miller et al. (2015) observed that population growth rate of viruliferous WCM increased with N fertilization and WSMV infection rates increased with increasing soil nitrate concentration in winter wheat. Timing of application can be manipulated in addition to the quantities of nutrient applied, but little work has been done in the manipulation of timing of nutrients application for the disease control (Walters and Bingham 2007).

Host plant resistance is an effective tool for WSMV and WCM management (Harvey et al. 1994; Holtzer et al. 1996; Zhang et al. 2015). Wheat cultivars resistant to WSMV and WCM have been developed (Fahim et al. 2012; Friebe et al. 2011; Martin et al. 2007; Zhang et al. 2015). However, resistant varieties are not available everywhere the disease occurs and have been proven to be a temporary solutions as resistance genes for WSMV are not effective at high temperatures (above 24° - 27° C) (Seifers et al. 2006, 2007). Also, WCM and WSMV have the capacity to evolve rapidly and overcome host resistance (Harvey et al. 1995; Seifers et al. 2007; Robinson and Murray 2013). Performance of available varieties when integrated with other cultural practices may give better result for WSMV management.

A better understanding of the interaction between host resistance and virus-vector dynamics with cultural practices is necessary for developing ecologically based management of WSMV. Previous studies have assessed the impacts of planting date, N fertilization, and use of resistant varieties on WSMV incidence using mechanical

inoculation of WSMV (Hunger et al. 1992; Miller et al. 2015; Talbert et al. 1996).

However, mechanical inoculation may not give accurate estimation of disease risk (Miller et al. 2014). Because of this knowledge gap, I evaluated the combined effect of planting date and winter wheat variety on WSMV incidence, yield, and grain quality using biological inoculation. I also assessed the joint effect of N application timing and winter wheat variety on WSMV incidence, yield, and grain quality using biological inoculation.

Materials and Methods

Site Description

Two complementary field experiments, one assessing the impact of planting date and wheat varietal selection on WSMV susceptibility and another evaluating the impact of N application timing on WSMV susceptibility on different winter wheat varieties were conducted during two growing seasons at same geographic location. Both experiments were conducted in 2013 - 2014 (2014 trial hereafter) and 2014 - 2015 (2015 trial hereafter) at the Montana State University Lutz Farm in Bozeman, MT, USA (45°48'16.29" N, 111°2'45.06" W, and 1408 m elevation). The soil type at the site was Hyalite-Beaverton complex and block dog slit loam with 0 to 4 percent slopes (Soil Survey Staff, NRCS, USDA, 2015).

Weather

Growing season temperature and precipitation as well as long term annual averages were recorded at the nearest weather station (Belgrade Airport weather station,

Western Regional Climate Center, approximately 9 km from the research site). Mean monthly temperature during 2014 growing season ranged from - 9.4 °C in February to 20.2 °C in July whereas in 2015 growing season it ranged from - 4.9 °C in December to 19.2 °C in July (Table 3.1). The long term annual mean temperature during 1941 to 2015 ranged from -7.6 °C in January to 19.3 °C in July (Table 3.1).

Planting Dates and Varietal Selection Experiment

This study assessed the impact of planting date on WSMV susceptibility on different winter wheat varieties. Treatments were assigned in a randomized complete split-plot design with six blocks. The main plot (18 m × 3 m) treatments were three planting dates: early planting (PD1), recommended planting (PD2), and late planting (PD3) (dates of planting listed below). Within main plots, five different winter wheat varieties were randomly assigned as 3 m × 2 m split plots. Main plots and split plots were separated by 5 m and 2 m wide buffer areas, respectively, and kept weed free using herbicide (listed below) and mechanically during the duration of the experiment. Wheat varieties were chosen based on their level of resistance to WSMV: Mace (resistant), Snowmass (resistant), TAM 112 (moderately resistant), Yellowstone (locally adapted, moderately susceptible), and Pronghorn (susceptible) (Baenziger et al. 1996; Graybosch et al. 2009; Haley et al. 2011; Rudd et al. 2014).

In the 2014 trial, winter wheat was seeded on 4 September (PD1), 19 September (PD2), and 2 October (PD3) of 2013. A Fabro (Swift Current, SK, Canada) no-till drill with seven double disc openers spaced 25.4 cm apart was used for seeding with a seeding

depth of 3.8 to 5.0 cm at the rate of 67 kg ha⁻¹. At sowing, we used a 17-17-17 (N-P-K) starter fertilizer at the rate of 100 kg ha⁻¹. We applied 401 kg ha⁻¹ to 430.4 kg ha⁻¹ urea in 2014 trials and 349.7 kg ha⁻¹ to 421.4 kg ha⁻¹ urea in 2015 trials at Feekes stage 2 of winter wheat in the fall depending upon residual nitrogen present in the soil in each block targeting 224 kg ha⁻¹ of nitrogen. During the growing season, the whole area was treated with clopyralid plus 2-4 D (Curtail ®) at the rate of 1.01 kg a.i. ha⁻¹ on 17 October 2013 to control broadleaf weeds. Buffer areas were sprayed with 2-4 D LV6 at the rate of 0.49 kg a.i. ha⁻¹ mixed with sulfosulfuron (Maverick® at the rate of 0.035 kg a.i. ha⁻¹) on 15 May 2014 by using a tractor with shielded sprayer to control drifting. Weeds within plots were control mechanically by hand weeding. To control stripe rust, plots were sprayed on 22 May and 3 June 2015 (Feekes stages at 10 to 10.1) with propiconazole and trifloxystrobin (Stratego®, 0.16 kg a.i. ha⁻¹) by using a CO₂ backpack sprayer with four XR Tee jet 8002VS nozzles.

In the 2015 trial, plots were seeded on 2 September (PD1), 18 September (PD2), and 2 October (PD3) of 2014 using the methods described above. For weed control, the area was treated with glyphosate (RoundUp Power Max® at the rate of 0.75 kg a.i. ha⁻¹) before planting on 18 August 2014. During the growing season, weeds were controlled using the methods described above.

Split plots were inoculated with WSMV by transplanting winter wheat (cv. Neeley) plants infested with viruliferous WCM grown in 15.2 cm pot in greenhouse following the methods described in Chapter 2 WCM and WSMV were continuously grown in growth chambers as stock populations of viruliferous WCM for inoculation using the methods described by Ito et al. (2012) and Miller et al. (2014). Individual wheat

plants were inoculated with a leaf segments containing 15 - 20 mites and plants were kept in greenhouse for two weeks before transplanting in the field. Within each split plot, five infested plants were placed in the center of the middle row at Feekes growth stage 3 - 4 of winter wheat on 14 September (PD1), 26 September (PD2), and 10 October 2014 (PD3) respectively. In 2015, all the split plots were bordered by simulated volunteer strips planted after wheat harvest around the area. The volunteer winter wheat strips (cv. Neeley) were seeded 13 August 2014 (seeding rate 44.8 kg ha⁻¹), and these strips were infested with mite infected plants grown in greenhouse to provide additional infestation of the experimental plots. We found mites on the volunteer wheat plants but the plants were not tested for WSMV. The middle row of volunteer strips was inoculated by transplanting winter wheat infested with viruliferous WCM as described above. The transplants provided the additional source of WCM and WSMV infection along with natural infection.

Nitrogen Application Timing Experiment

This study evaluated the impact of N application timing on WSMV susceptibility of five winter wheat varieties. Treatments were assigned in a randomized complete split-plot design with six blocks. Blocks were arranged in an east-west direction to accommodate the slope of the field. Main plot treatments (2 m × 31.5 m) were randomly assigned to one of five winter wheat varieties varying in their resistance to WSMV as described in planting date experiment above. Each variety was seeded in five rows which were split into six split-plots of 4 m × 2 m where six combinations of N application

timing (fall, early spring and late spring) and WSMV inoculation status (inoculated and control) were randomly assigned in each split-plot. Main plots and split plots were separated by a 2 m and 1.5 m buffer area, respectively.

Winter wheat varieties were seeded on 19 and 18 September in 2013 and 2014 for the 2014 trial and 2015 trial, respectively, using the methods described above. WSMV split plots were inoculated by transplanting winter wheat infested with viruliferous WCM as described above on 15 October 2013 and 14 October 2014 for the 2014 trial and 2015 trial, respectively. Stripe rust and weeds within plots and buffer areas were controlled as described above. We applied 359.8 kg ha⁻¹ to 393.4 kg ha⁻¹ urea in 2014 trials as fall application (15 October 2013; Feekes stage 3), early spring (12 May 2014; at Feekes stage 6) and late spring (21 May 2014; Feekes stage 8-9). Similarly, 341.9 kg ha⁻¹ to 427 kg ha⁻¹ urea was applied in 2015 trials as fall application (16 October 2014; Feekes stage 3), early spring (12 May 2015; at Feekes stage 6-7) and late spring (2 June 2015; Feekes stage 9-10) depending upon residual nitrogen present in the soil in each block targeting 224 kg ha⁻¹ of nitrogen.

Sampling and Detection of WSMV

In both experiments, thirty wheat flag leaves were sampled by a stratified random sampling from the second, third, fifth and sixth rows within each split-plot at Feekes growth stages 10.1 to 10.5 (heading to flowering stages) to detect WSMV. During sampling, the inoculated row (fourth row) and two outer rows (first and seventh rows) were not sampled to avoid the chances of sampling inoculated plants and edge effects.

However, in 2015, we sampled all the rows except two outer rows of planting date experiments.

All leaves were processed individually with indirect enzyme-linked immunosorbent assay (ELISA) as described by Ito et al. (2012). Six to eight negative control samples consisting of healthy wheat grown in greenhouse were systematically distributed in the wells of a plate to reduce bias in optical density caused by position of samples in the plate. There was one positive control and one buffer wells in each plate. The mean and standard deviation of the negative control on each plate were used to set a probabilistic optical density threshold at three standard deviations above the mean. Samples above this threshold were considered infected with WSMV (Miller et al. 2014).

Yield measurement

Split-plots were harvested by using plot combine (Classic model - Wintersteiger AG, Utah, USA) and seeds were cleaned and weighed. Yields were converted into MT ha⁻¹ prior to analysis. Grain quality measures including protein, percentage moisture, and test weight were conducted at the Cereal Quality Laboratory, Plant Science and Plant Pathology, Montana State University, MT using standard procedures.

Statistical Analysis for Planting Date and Varietal Selection Experiment

To assess the relationship between WSMV incidence and planting date and wheat variety, we fitted a generalized linear mixed-effects model with a binomial distribution. All analyses were performed in R version 3.0.1 (R- Development Core Team, 2014)

using the lme4 package (Bates et al. 2015). WSMV incidence was measured as the number of plants infected divided by the total number of plants sampled from each split-plot as a response variable. The data of zero infection out of total samples (probability = 0) were transformed as the logit function is undefined with zero values (Ramsey and Schafer 2002). During transformation, sample size of data that had probability 0 was doubled and added one success (Agresti and Caffo 2000; Agresti and Coul 1998; Warton and Hui 2011). Results are presented as the untransformed probability of WSMV incidence for clarity. The relationships between WSMV infection, planting date, and winter wheat variety was tested using competing richness models. Planting date, winter wheat variety, and trial (year) were modeled as fixed effects. The unique combination of year and planting date nested within block was considered a random effect. The trial was modeled as a fixed effect to test for consistency of results between years. The best-fit model was selected with the lowest Akaike's information criterion (AIC) values and that model was selected for interpretation (Bolker et al. 2009; Burnham and Anderson 2002; Garrett et al. 2002).

A linear mixed model was used to assess differences in yield, protein content, and percentage moisture across planting dates and winter wheat varieties. Planting date, winter wheat variety, and trial were modeled as fixed effects. The unique combination of year and planting date nested within block was considered a random effect. Model selection was described above. Outliers were removed from the selected model by using `romr.fnc` function from `LMERConvenienceFunctions`, R package, where an outlier was defined as any observation from the selected model with standardized residuals greater than 2.5 standard deviations from the mean (Tremblay and Ransijn 2013). Homogeneity

of variance and normality were visually evaluated by using residual and Q-Q plots. Yield data in the response phase were natural log-transformed to improve the normality, but untransformed data are presented in the results for clarity. Protein, moisture and test weight data did not require transformation. General linear hypothesis test (glht) with Tukey multiple comparisons in MULTCOMP package were used to compare difference among planting dates and variety (Hothorn et al. 2015). Packages ‘sciplot’ (Morales 2012) and ‘ggplot2’ (Wickham 2009) were used to build graphs.

Statistical Analysis for N Application Timing and Varietal Selection Experiment

To assess the relationship between WSMV incidence and timing of N application across winter wheat varieties, we utilized similar data transformation and statistical analysis to that described above. Winter wheat variety, N application timing, and trial were modeled as fixed effect whereas the combination of each year winter wheat variety nested within block was considered a random effect.

Results

Planting Date and Winter Wheat Variety Effects on WSMV

WSMV incidence associated with planting date and variety was consistent between years (planting date \times variety \times year, $\chi^2 = 4.3$, df = 8, $P = 0.82$). However, difference in WSMV incidence among planting dates depended on winter wheat varieties (planting date \times variety, $\chi^2 = 32.5$, df = 8, $P < 0.01$) as well as it was dependent on winter wheat variety and year (variety \times year, $\chi^2 = 11.9$, df = 4, $P = 0.01$). Observed difference

in WSMV incidence among planting dates did not differ by year (planting date \times year, $\chi^2 = 0.2$, $df = 2$, $P = 0.87$). This result indicates that planting date and variety interaction consistent among years and variety and year interaction did not vary by planting dates. Averaged over years, Pronghorn had a higher WSMV incidence (10%, $P < 0.01$) in PD1 than PD2 (3%) and PD3 (2%). There was no difference in WSMV incidence between planting dates in Mace, Snowmass, TAM 112 and Yellowstone ($P > 0.5$ in all contrast) (Fig. 3.1).

Crop yield among planting dates and varieties were consistent between years (planting date \times variety \times year, $\chi^2 = 12.3$, $df = 8$, $P = 0.13$). Observed difference in yield between planting dates did not vary by winter wheat variety (planting date \times variety, $\chi^2 = 7.1$, $df = 8$, $P = 0.51$). However, yield differed among planting dates ($\chi^2 = 31.96$, $df = 2$, $P < 0.01$) but yield was similar across varieties ($\chi^2 = 6.4$, $df = 4$, $P = 0.16$). Average yield was consistent between years ($\chi^2 = 0.07$, $df = 1$, $P = 0.78$). Across planting dates, PD3 (3.3 MT ha⁻¹) had a lower average yield than PD2 (4.5 MT ha⁻¹, $P = 0.02$) and PD1 (5.17 MT ha⁻¹, $P < 0.01$), but there was no difference in yield between PD1 and PD2 ($P = 0.61$) (Fig: 3.2).

Averaged protein content had an increasing trend in both years from PD1 to PD3, which ranged from 11.9 % to 14.6 % (Table 3.2). The observed difference in protein content among planting dates and winter wheat variety differed by years (planting date \times variety \times year, $\chi^2 = 24.9$, $df = 8$, $P < 0.01$). In 2014, Mace and Pronghorn had higher protein content in PD3 than PD1 ($P < 0.01$) but PD1 and PD2 ($P = 0.25$) as well as PD2 and PD3 had similar protein content ($P = 0.99$) on these two varieties. Snowmass and Yellowstone had higher protein content in PD3 than PD1 ($P < 0.01$) and PD2 ($P < 0.01$),

but PD1 and PD2 had similar protein content ($P = 0.99$) on them. In TAM 112, PD1 (10.7 %) had lower protein content than PD2 (12.6 %, $P < 0.01$) and PD3 (14.8 %, $P < 0.01$). In 2015, there were no differences in protein content between planting dates among winter wheat varieties ($P > 0.6$ in all contrast) except Yellowstone. In Yellowstone, PD1 (11.5%) had lower protein content than PD2 (12.9 %, $P < 0.01$), and PD3 (13.4 %, $P < 0.01$) (Table 3.2).

There was a general decreasing trend of test weight from PD1 to PD3, which ranged from 82.9 Kg hl⁻¹ to 80.0 Kg hl⁻¹ in 2014, and 80.1 Kg hl⁻¹ to 72.9 Kg hl⁻¹ in 2015. Test weights associated with planting dates depended on winter wheat varieties and years (planting date \times variety \times year, $\chi^2 = 19.8$, df = 8, $P < 0.01$). However, observed difference on test weight between planting date did not depend on winter wheat varieties (planting date \times variety, $\chi^2 = 11.9$, df = 8, $P = 0.15$) as well as between varieties and years (variety \times year, $\chi^2 = 2.9$, df = 4, $P = 0.56$). In 2014, Mace had lower test weight in PD3 (78.7 Kg hl⁻¹) than PD1 (81.3 Kg hl⁻¹, $P < 0.01$) and PD2 (80.2 Kg hl⁻¹, $P = 0.01$), but PD2 and PD1 had similar test weight ($P = 0.29$). TAM 112 in 2014 had higher test weight in PD1 (83.8 Kg hl⁻¹) than PD3 (82.0 Kg hl⁻¹) (Table 3.2). There was no difference in test weight between planting dates in Pronghorn, Snowmass and Yellowstone ($P > 0.5$ in all contrast) in 2014. In 2015, test weight was similar between planting dates in all winter wheat variety except Snowmass (Table 3.2). In Snowmass, PD1 (80.1Kg hl⁻¹) had higher test weight than PD2 (74.8Kg hl⁻¹, $P < 0.01$), but PD2 and PD3 (76.7Kg hl⁻¹, $P = 0.98$), had similar test weight.

N Application Timing and Winter
Wheat Variety Effect on WSMV

The WSMV incidence associated with N application timing, winter wheat varieties, and year did not depend upon WSMV status (inoculated and control plots) (N application \times variety \times year \times status, $\chi^2 = 3.9$, $df = 8$, $P = 0.85$). The observed difference on WSMV incidence among N application timing marginally depended on winter wheat varieties (N application \times variety, $\chi^2 = 12.3$, $df = 8$, $P = 0.06$). WSMV incidence varied between N application time depended on year (N application \times year, $\chi^2 = 14.3$, $df = 2$, $P < 0.01$) and it was depended on variety and year as well (variety \times year, $\chi^2 = 14.5$, $df = 4$, $P < 0.01$).

In 2014, WSMV incidence was similar between N application timing in resistant varieties; Mace, Snowmass and TAM 112 (Fig. 3.3). However, Pronghorn had higher WSMV incidence when N applied in early spring (12 %,) than N applied in fall (5 %, $P = 0.03$). But, there was similar WSMV incidence when N applied in early and late spring ($P = 0.14$). Likewise, Yellowstone had higher WSMV incidence when N was applied in early spring (15 %) than late spring (6 %, $P < 0.01$). But, there was similar WSMV incidence when N applied in early spring and fall (9%, $P = 0.27$) and between fall and late spring ($P = 0.53$). In 2015, there was no difference in the incidence of WSMV among N application timing in Mace, Snowmass, TAM 112 and Yellowstone except Pronghorn. Pronghorn had higher WSMV incidence in late spring (7%) than early spring (2%, $P = 0.02$), but WSMV incidence was similar when N applied on fall and early spring ($P = 0.99$).

There was no difference in yield due to N application time in both year and yield ranged between 6.2 MT ha⁻¹ and 6.4 MT ha⁻¹. Average yield associated with N application timing, variety, and year did not depend on WSMV status (N application × variety × year × status, $\chi^2 = 4.1$, df = 8, $P = 0.84$). Observed difference in yield between N application timing did not depend on winter wheat varieties (N application × variety, $\chi^2 = 6.2$, df = 8, $P = 0.64$) or year (N application × year, $\chi^2 = 0.12$, df = 2, $P = 0.94$). Yield differed among winter wheat varieties ($\chi^2 = 37.7$, df = 4, $P < 0.01$) but not with N application timing ($\chi^2 = 2.6$, df = 2, $P = 0.2$). Averaged across varieties, Yellowstone had a higher yield than Mace ($P < 0.01$) regardless of nitrogen application timing (Fig. 3.4).

Average protein content associated with N application timing, variety, and year was consistent between inoculated and control plots (N application × variety × year × status, $\chi^2 = 13.1$, df = 8, $P = 0.08$). However, observed difference in protein content among N application timing depended on years (N application × year, $\chi^2 = 202.2$, df = 2, $P < 0.01$) as well as winter wheat variety (N application × variety, $\chi^2 = 60.7$, df = 8, $P < 0.01$). There was a general increasing trend in protein contents from fall to later spring N application which ranged from 11 % to 14.5 % (Table 3.3). In 2014, Mace, Snowmass and Yellowstone had higher protein content when N applied in late spring than fall ($P < 0.01$) and early spring N application ($P < 0.01$), but fall and early spring N application had similar protein content on these varieties ($P = 0.99$). Pronghorn and TAM 112 had higher protein content when N applied in late spring than fall ($P < 0.01$) and early spring N application ($P < 0.01$). In these latter two species protein content was higher when N applied in early spring than fall ($P < 0.01$). In 2015, there was no difference in protein content between N application timing in Mace, Pronghorn and Snowmass ($P > 0.2$ in all

contrast). However, TAM 112 had higher protein content when N applied on late spring (12.7 %) than N applied in fall (11.4 %, $P < 0.01$). was applied in the fall and early spring ($P = 0.6$). In Yellowstone, protein content was higher when N applied in early spring (12.5 %) than fall (11.4 %, $P < 0.01$) and late spring N application (11.6 %, $P < 0.01$) (Table 3.3).

Average test weight associated with N application timing, variety, and year was consistent between control and inoculated plots (N application \times variety \times year \times status, $\chi^2 = 5.5$, $df = 8$, $P = 0.7$). Observed difference in test weights between N application timing depended on winter wheat varieties and year (N application \times variety \times year, $\chi^2 = 23.9$, $df = 8$, $P < 0.01$). In 2014, there was similar test weight between N application timing within variety ($P > 0.2$) (Table 3.3). In 2015, there was similar test weight between N application timing in Mace, Pronghorn and Yellowstone. However, in Snowmass, late spring N application had higher test weight (80.7 kg hl^{-1}) than fall (78 kg hl^{-1} , $P < 0.01$) and early spring N application (79 kg hl^{-1} , $P = 0.02$). In TAM 112, late spring N application (82.5 kg hl^{-1}) had higher test weight than fall N application (80.4 kg hl^{-1} , $P < 0.01$), but there was similar test weight when N applied on late spring and early spring (81.7 kg hl^{-1} , $P = 0.23$) as well as fall and early spring ($P = 0.99$).

Discussion

Understanding the impact of agronomic practices on WSMV incidence, wheat yield, and grain quality in field conditions can contribute to the design of ecologically based disease management strategies. Although this study had low WSMV incidence, we

found useful results on the impact of planting date and N application timing on disease incidence, yield and grain quality of winter wheat.

Wheat streak mosaic virus incidence differed due to timing of winter wheat planting. There was almost no infection in resistant varieties. For the susceptible variety Pronghorn, incidence of WSMV was higher in the early planting date (PD1) than recommended (PD2) and late (PD3) planting dates. PD1 coincided with the harvest period for cereal crops and maturation of several alternative host species in the area. During early fall, viruliferous mites move from surrounding cereal fields and/or from alternative hosts and infest the newly emerged crops (Nault and Styer 1969, Thomas et al. 2004). Hunger et al. (1992) and Wills (1984) also found that early planted wheat was infected at higher rates by WSMV compared to later planting dates. The study of the influence of alternative hosts during fall explored the peak WCM infestation and WSMV infection at the beginning of the fall (Chapter 2). According to the results, if winter wheat emerged during weeks of high WCM immigration, typically before and during mid-September, this increases the risk of WCM infestation and WSMV infection of winter wheat. Therefore, early planting of a susceptible winter wheat variety is not recommended practice when disease potential is high. However, resistant varieties showed adaption to Montana climates under low disease pressure.

Yield and grain quality including protein differed across winter wheat planting dates and varieties. Yields of PD1 and PD2 did not differ, but averaged across planting date, PD3 had lower yield than PD1 and PD2. Incidence of WSMV was low in both years, and was unlikely to affect yield. Differences in yield were likely due to fall establishment and temperature during grain filling period. Porter and Gawith (1999) and

Thiry et al. (2002) found that late planting of winter wheat resulted in fewer tillers or head leading to low yield. Plants suffered from high temperatures during grain filling that increased senescence and decreased grain filling duration. The results of high grain protein and test weight of PD3 might be due to the high temperature stress during grain fill compared to PD1 (Jones and Olson-Rutz 2012).

Nutrient availability can affect disease severity (Huber et al. 1999), but the magnitude of this impact depends upon the type and concentration of the nutrient as well as the specific patho-system (Dordas 2008). In this study, WSMV incidence was low and incidence was similar between resistant varieties but susceptible varieties had higher incidence than resistant. Results of 2014, the year with relatively higher WSMV incidence, indicated that early spring N application can result in increased WSMV incidence in susceptible variety compared to fall in Pronghorn and late spring application of N in Yellowstone. This result indicates that N application may increase WSMV incidence in a susceptible variety when N is applied during early growth stages of winter wheat. However, it is not consistent over years and fall application did not alter the risk relative to late spring in most cases. Additional studies with higher disease pressure will be needed to confirm this observation. From the epidemiological perspective, N fertilization may increase the risk of WSMV infection and WCM infestation as it increases the population growth rates of viruliferous WCM (Miller et al. 2015).

Nitrogen application timing had no effect on yield, but grain protein increased with later N application dates. Brown and Petrie (2006) also found that fall and spring nitrogen application on winter wheat resulted in similar yields that might be due to higher kernel weight (Sadaphal and Das 1966; Gravelle et al. 1988). Similar yields across N application

time suggests that growers get same yield when they apply N during different times of the growing season, as long as N is not lost due to volatilization (Jones and Olson-Rutz, 2012). The results of high grain quality when N was applied during the late spring may be due to the application of N during heading. N application during and after heading tend to increase protein content of grain (Brown and Petrie 2006; Jones and Olson-Rutz, 2012). Amount of N can be split to increase yield and grain quality by modifying the application timing within a single field.

From the disease management prospective, if a field is infected with WSMV, modifying the timing of N application can reduce the risk of WSMV spread. This is due to the fact that WCM reproduce more quickly on adequately fertilized wheat plants (Miller et al. 2015) and N application could increase viruliferous mite population as viruliferous WCM reproduce faster than non-viruliferous (Siriwetwivat 2006).

Management of WSMV relies on integrated approaches due to complexity of the patho-system (Navia et al. 2013). This study showed that planting winter wheat in mid to late September reduces the risk of WSMV. But, resistant variety for example TAM 112 AND Snowmass provides similar yield to locally adapted variety, Yellowstone and had low risk of WSMV infection at least for low disease pressure. TAM 112 and Snowmass can be used as WSMV resistant varieties in Montana climates. In the areas of high WSMV risk, along with planting dates, producers can split the timing and amount of N application to reduce the probable risk of WSMV epidemics. Collectively, planting date, N application timing and use of resistant varieties contributes to effective sustainable WSMV management.

Table 3.1. Average monthly temperature and total monthly cumulative precipitation during winter wheat growing season (August to July) from 2013 to 2015 at Belgrade Airport, approximately, 9 km from study area

Months	Temperature (°C)			Precipitation (mm)		
	2013-2014	2014 -2015	LTA*	2013-14	2014-15	LTA*
August	19.9	17.9	18.5	25.4	74.4	28.7
September	14.8	12.7	12.8	62.7	33.0	33.0
October	4.1	8.4	6.6	9.1	9.9	26.7
November	0.1	-3.8	-1.2	9.1	24.1	19.6
December	- 8.19	-4.9	-6.4	8.4	14.9	13.9
January	-3.0	-4.3	-7.6	7.4	5.6	14.2
February	-9.4	0.1	-4.5	16.8	3.8	11.2
March	0.8	4.6	-0.4	37.8	11.9	22.9
April	5.8	5.5	5.5	23.9	45.5	33.8
May	11.0	10.3	10.4	39.1	84.6	56.9
June	13.9	18.1	14.8	103.6	15.2	63.7
July	20.2	19.2	19.3	9.6	45.2	28.2
Total				353	368.1	

*Long-term average, are based on 75 years of data (1941 - 2015) mean temperature (°C), and precipitation (mm) at 9 km from the study area (Data source: Belgrade Airport weather station, Western Regional Climate Center)

Table 3.2: Average grain quality of three planting dates across winter wheat varieties

Protein content (%)						
Variety	Year					
	2014			2015		
	PD1^{zy}	PD2^{zy}	PD3^{zy}	PD1^{zy}	PD2^{zy}	PD3^{zy}
Mace	13.2 a	13.9 ab	15.3 b	11.7 a	12.6 a	12.2 a
Pronghorn	12.9 a	13.5 ab	15.0 b	12.4 a	13.3 a	13.1 a
Snowmass	10.9 a	11.8 a	13.9 b	11.5 a	12.9 a	12.1 a
TAM 112	10.7 a	12.6 b	14.8 c	11.4 a	13.7 a	12.5a
Yellowstone	11.9 a	12.6 a	14.2 b	11.5 a	12.9 b	13.4 b
Test weight (Kg hl⁻¹)						
Mace	81.3 b	80.2 b	78.7 a	76.0 a	74.4 a	75.6 a
Pronghorn	82.8 a	82.6 a	81.7 a	79.3 a	77.4 a	77.6 a
Snowmass	83.1 a	82.6 a	81.8 a	80.1 b	74.8 a	76.7 ab
TAM 112	83.8 b	82.9 ab	82.0 a	78.5 a	75.3 a	78.4 a
Yellowstone	80.9 a	80.8 a	80.0 a	77.0 a	76.6 a	72.9 a

^z PD1 is early planting date (Sept 4 and 2 in 2014 and 2015 respectively), PD2 is recommended planting date (Sept 19 and 18 in 2014 and 2015 respectively) and PD3 is late planting date (Oct 2 in both years)

^y Different letters within variable and year indicate significant differences between planting dates within winter wheat variety (P < 0.05)

Table 3.3: Average grain quality of winter wheat varieties on fall, early spring, and late spring application of nitrogen

Variety	Protein content (%)					
	Year					
	2014			2015		
	Fall ^z	Early spring ^z	Late spring ^z	Fall ^z	Early spring ^z	Late spring ^z
Mace	12.7 a	12.8 a	13.9 b	11.7 a	12.1 a	11.7 a
Pronghorn	12.4 a	13.3 b	14.5 c	12.1 a	12.3 a	12.2 a
Snowmass	11.3 a	12.2 a	13.7 b	11.4 a	12.0 a	11.8 a
TAM 112	11.3 a	12.7 b	14.5 c	11.4 a	12.4 b	12.7 b
Yellowstone	11.0 a	11.6 a	13.1 b	11.4 a	12.5 b	11.6 a
Test weight (Kg hl ⁻¹)						
Mace	80.9 a	81.2 a	80.6 a	78.0 a	77.8 a	78.3 a
Pronghorn	82.5 a	82.7 a	82.6 a	79.5 a	80.8 a	81.1 a
Snowmass	82.5 a	82.3 a	82.5 a	78.0 a	79.0 a	80.7 b
TAM 112	83.5 a	82.8 a	82.8 a	80.4 a	81.7 ab	82.5 b
Yellowstone	80.4 a	80.5 a	80.2 a	78.3 a	78.4 a	79.7 a

^z Different letters within year and variable indicate significant differences between N application timing within winter wheat variety (P < 0.05)

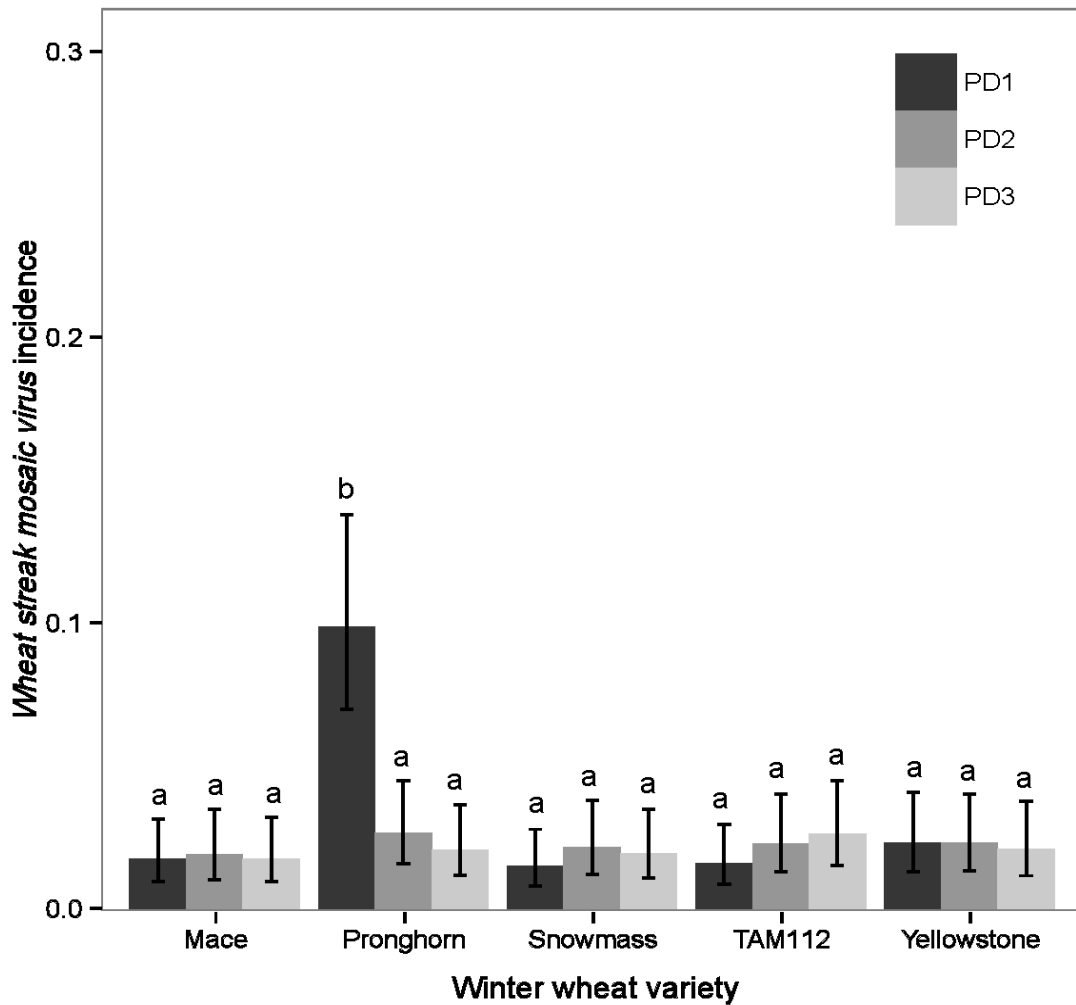


Fig. 3.1. Mean probability of *Wheat streak mosaic virus* (WSMV) incidence on winter wheat varieties between planting dates, data from 2014 and 2015 is combined. PD1 is early planting date, PD2 is recommended planting date and PD3 is late planting date. WSMV infection was detected using indirect enzyme-linked immunosorbent assay (ELISA) at Feekes growth stage 10.1 to 10.5. Different letters within winter wheat variety indicate significant differences between planting dates ($P < 0.05$).

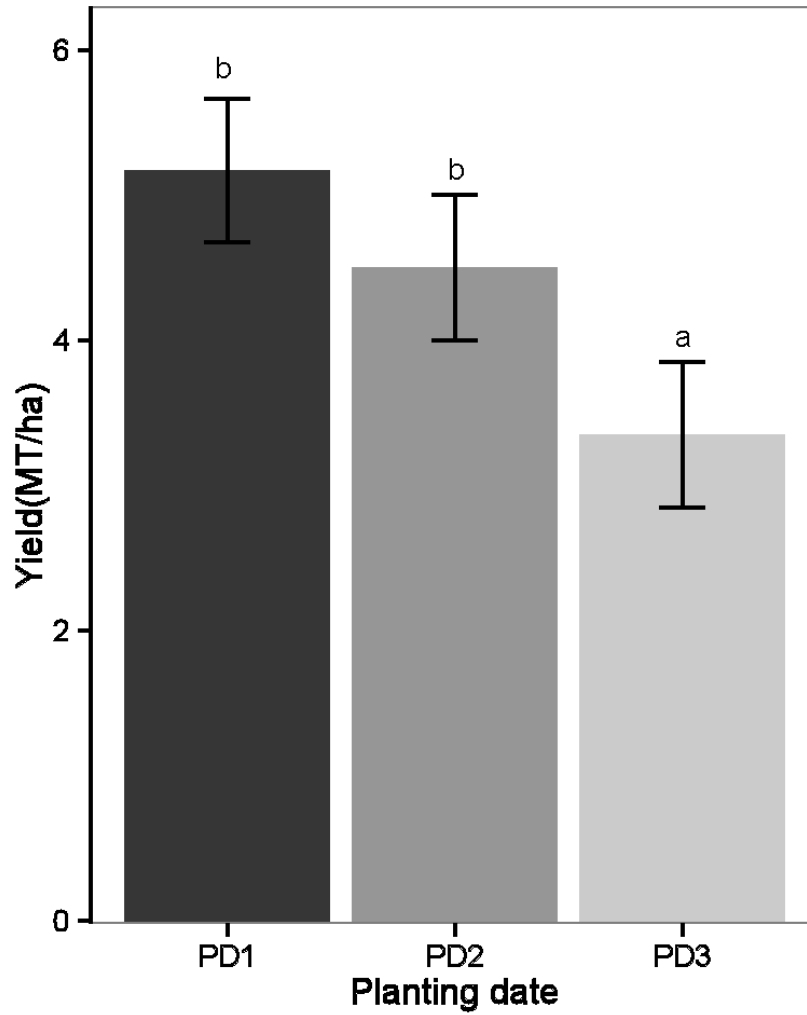


Fig. 3.2. Average yield (MT ha⁻¹) of winter wheat planted on three planting dates. Data from 2014 and 2015 as well as varieties is combined. PD1 is early planting date, PD2 is recommended planting date, and PD3 is late planting date. Different letters indicate significant differences between planting dates (P < 0.05).

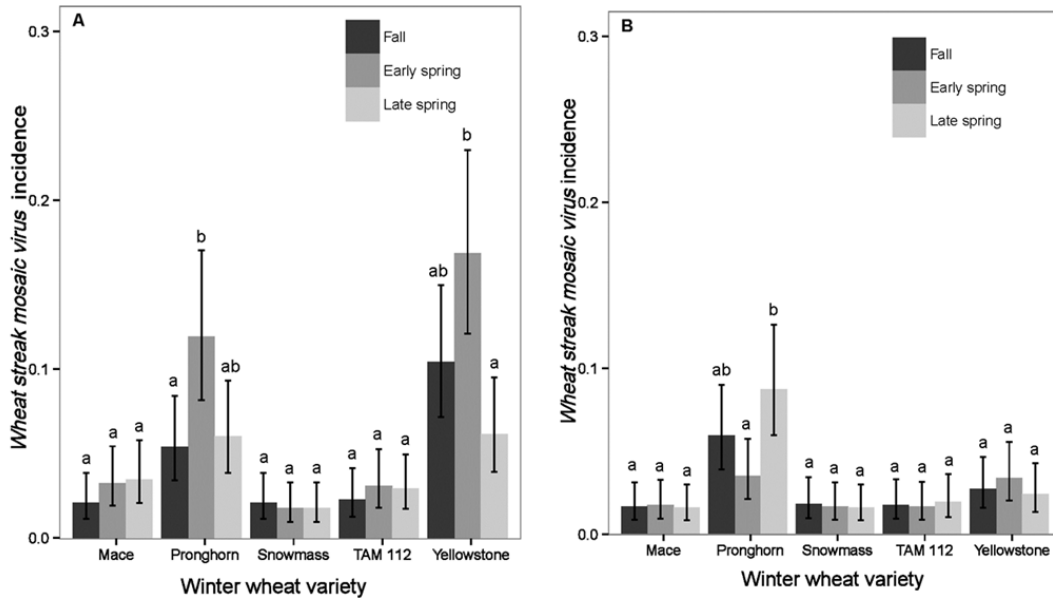


Fig. 3.3. Mean probability of *Wheat streak mosaic virus* (WSMV) incidence on winter wheat varieties in fall, early spring, and late spring N application timing across for **A**, 2014; **B**, 2015. WSMV incidence was detected using enzyme-linked immunosorbent assay (ELISA) at Feekes growth stage 10.1 to 10.5. Different letters within variety indicate significant differences between N application timing ($P < 0.05$).

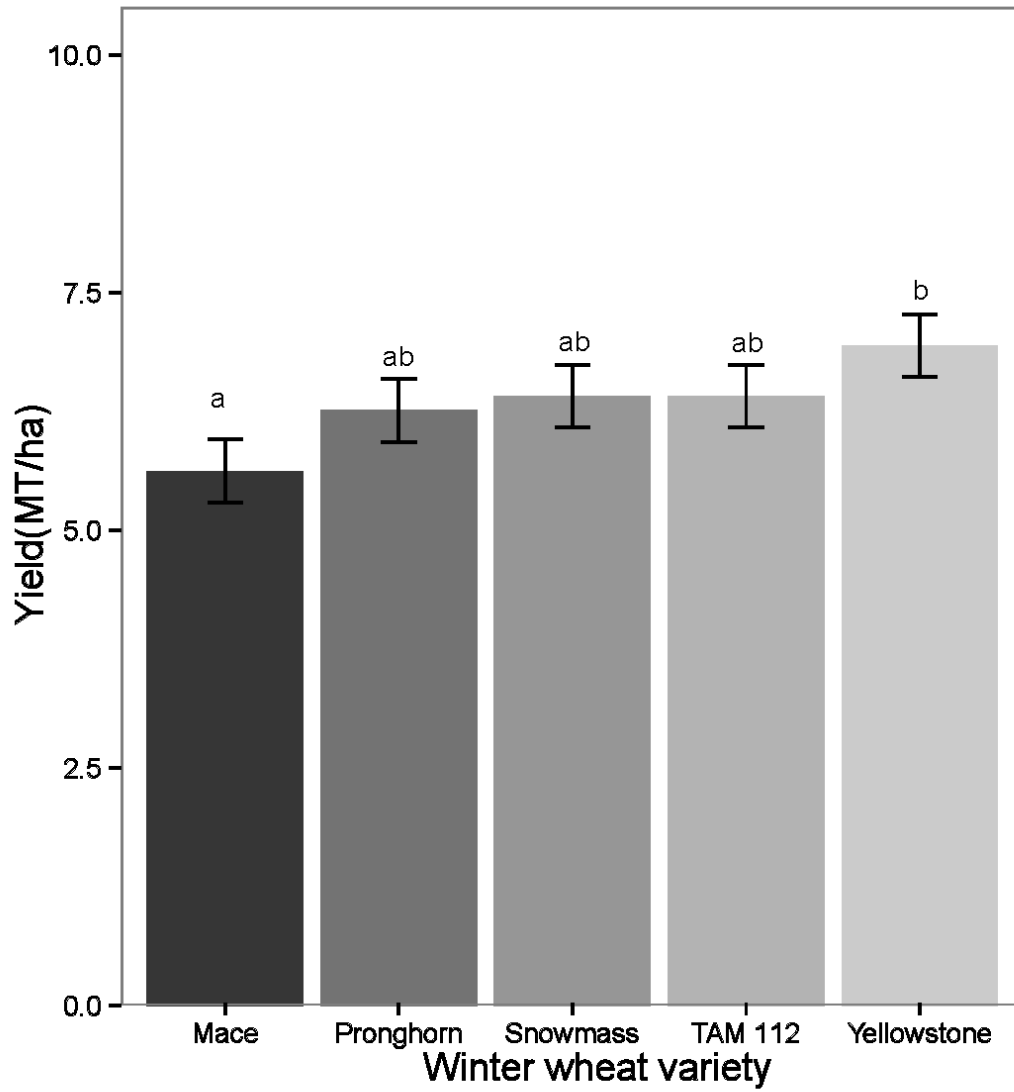


Fig. 3.4. Average yield (MT ha^{-1}) of winter wheat varieties, yield was pooled across N application timing.

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CHAPTER FOUR

IMPACT OF CROPPING SYSTEM AND COVER CROP TERMINATION METHODS ON DISEASES OF WHEAT AND LENTILS

Introduction

Summer fallow is being replaced with cover crops in some locations of the Northern Great Plains (NGP) (Tanaka et al. 2010). Cover crops are single species or mixtures of plant species established for temporary soil cover, improvement of soil physical and chemical properties, enhancement of nutrient cycling (Delgado et al. 2007), and pest management (Altieri and Nicholls 1999; Gallandt et al. 1999; Sulc and Franzluebbers 2014; Tillman et al. 2012). If leguminous species are used as cover crops, available soil nitrogen is increased due to biological nitrogen fixation (Snapp et al. 2005). To maximize their benefits, cover crops need to be terminated in a timely manner to avoid excessive water loss from the soil in the semi-arid environments of the NGP (Zentner et al. 2004).

Tillage or herbicides are the most common methods of cover crop termination in the NGP (Foster 1990). However, intensive tillage could lead to unintended consequences such as increased soil erosion (Greer et al. 2006), air and water pollution (Logan et al. 1991; Papendick and Parr 1997), and decreases in carbon sequestration (West and Marland 2002), soil moisture, organic matter (Holland 2004), relative density of beneficial arthropods (Kladivko 2001; Lalonde et al. 2012), and diversity of soil

micro-organisms (Roper and Gupta 1995). Similarly, excessive reliance on pesticides can have consequences including the selection of pesticide resistance pest biotypes, buildup of pesticide residues in soil and water, non-target effects on beneficial organisms, and pest resurgence (Calderbank 1989; Guedes et al. 2016; Powles and Yu 2010).

Consequently, there is a growing interest in alternative termination methods that enhance agricultural sustainability in dryland farming.

The use of livestock for the termination of cover crops may increase nutrient cycling, help weed management, provide revenue through production of food and fiber, and represent an alternative approach to reduced tillage (Entz et al. 2002; Franzluebbers 2007; Russelle et al. 2007; Thiessen Martins and Entz 2011). Cattle grazing of cover crop under drought conditions have been shown to have a net economic advantage in a no-till wheat cropping system compared to conventional tillage (Franzluebbers and Stuedemann 2014). However, livestock integration can lead to soil compaction and represents an additional capital investment (Franzluebbers and Stuedemann 2014; Undersander et al. 2002). Targeted grazing with small ruminants such as sheep may reduce insect pests such as wheat stem sawfly (Goosey et al. 2005). Additionally, the distribution of feces and urine by small grazers is more even in the field when compared to cattle (Abaye et al. 1997). Furthermore, sheep-crop integration is well-suited to the NGP as animals are easily transported and available in the region (NASS 2015). While impact of cover crops and sheep grazing on weed communities, carabid beetle communities, wheat stem sawfly larval mortality have been studied (Barroso et al. 2015; Miller et al. 2014; McKenzie et al. 2016; Goosey et al. 2005); less is known about their impact on plant disease incidence and severity in dryland farming systems.

There is no consensus in past research regarding the incidence and severity of wheat pathogens concerning the effects of organic system compared to conventional. For example, rust, powdery mildew, and leaf blotch were less severe in organically than conventionally managed systems (van Bruggen 1995). Suppression of take-all disease of wheat was greater in the organic soils than conventional soils (Hiddink et al. 2005). Disease incidence common root rot (*Cochliobolus sativus*) was decreased and Fusarium root rot (*Fusarium* spp.) was increased under reduced tillage wheat fields compared to conventional tillage but there was no difference in leaf spot diseases (Bailey et al. 2001). Disease severity of Rhizoctonia root rot and Pythium root rot did not differ between tilled and no-till plots (Schroeder and Paulitz 2006; Smiley et al. 1996). Disease impact can be altered by other factors within system like crop diversity within rotation.

The NGP has enormous potential to expand organic production (Carr et al. 2011; Stofferahn 2009). However, the potential risk of pest resurgence upon transition from conventional to organic farming is a critical hurdle to expanding organic production (Chellemi et al. 2013). Changes in agricultural production systems can have different impacts on the intensity and severity of plant diseases (Smukler et al. 2008) and disease management options are limited for organic growers (McSorley 2002; van Bruggen and Termorshuizen 2003). Knowledge of potential risk factors during the transition period of organic production would reduce barriers for organic growers to initiate the transition process (Hanson et al. 2004). It is essential to understand how agronomic practices impact pests before their implementation. Therefore, this study assessed the impact of targeted sheep grazing, tillage, and herbicide cover crop termination methods on disease

incidence and severity in organic and conventional production systems during the transition to organic and the first years of the organic phase.

Materials and Methods

Site Description

The experiment was conducted at the Fort Ellis Agricultural Research Station of Montana State University, Bozeman, MT from 2013 to 2016. This study site is located at 45°40'02N, 110°58'38W at an elevation of 1496 m and approximately 8 km east of Bozeman. The long term annual mean temperature during 1892 to 2016 ranges from -5.5 °C in January to 19.1 °C in July and annual precipitation averages 469.1 mm (Table 4.1). The soil at the site is Blackmore silt loam (a fine-silty, mixed, superactive, frigid Typic Argiustoll) with a pH of 6.7 at 0 to 15cm depth (Barsotti et al. 2013).

Experimental Design

The entire experimental site was used for pasture for ten years before 2004. From 2004 to 2008, wheat was grown as continuous wheat-fallow system in a randomized split-plot design (Lenssen et al. 2013). From 2009 to 2012, the wheat-fallow rotations were replaced with either a continuous alfalfa (*Medicago sativa* L.) crop or a three year rotation of spring wheat (*Triticum aestivum* L.) - pea (*Pisum sativum* L.) / barley (*Hordeum vulgare* L.) hay mixture-fallow, maintaining the same fallow treatments (Barsotti et al. 2013). The experiment described here was a split-plot design that began in July 2012 with three management systems assigned as main-plot levels (0.55 ha): sheep

grazed-organic, tilled-organic, and chemical no-tillage for a total of 9 main plots with three replications. Each main plot was split into five split-plots (0.11 ha, 13 × 90 m in size) where a five year cropping sequence was established: safflower (*Carthamus tinctorius* L.) under-sown with sweet clover (*Melilotus officinalis* L.) (Y1), sweet clover green manure (Y2), winter wheat (Y3), lentil (*Lens culinaris* Medik) (Y4); and winter wheat (Y5) were randomly assigned to split-plots and each phase was present in each year. Main plots and split-plots were separated by 10 m and 1 m of alley, respectively. Disease assessments focused on disease incidence and severity of winter wheat (Y3), lentils (Y4), and winter wheat (Y5).

Crop Seeding and Crop Management

Organic plots received no synthetic inputs or fertilizers. In the tilled-organic plots, tillage was used for seedbed preparation, weed control, and cover crop termination. In grazed-organic plots, sheep were used to terminate cover crops and for weed control. In the chemical no-till systems, herbicides were used to terminate cover crops and for weed control. The seeding density on both grazed and tilled organic sub-plots was double that of conventionally managed plots, as a typical weed control tactic in organic systems. Winter wheat (cv. Yellowstone), was seeded on 23 Sep 2012, 24 Sep 2013, 17 Sep 2014, and 23 - 24 Sep 2015. Both tilled and grazed organic sub-plots were seeded at the rate of 123 kg ha⁻¹ (500 plants m⁻²) with 13 cm row spacing whereas chemical no-till sub-plots were seeded at the rate of 62 kg ha⁻¹ (250 plants m⁻²) with 25 cm row spacing by using a Fabro (Swift Current, SK, Canada) disk seeder. Wheat seed used for chemical no-till

split-plots were treated with 0.25 ml a.i. kg⁻¹ of CruiserMaxx Vibrance Cereals (sedaxane, difenoconazole, mefenoxam and thiamethoxam) and 0.11 ml a.i. kg⁻¹ Dividend XL RTA (difenoconazole and metalaxyl-M) fungicides in all years.

Supplementary Table 4.S1 summarizes the agronomic practices used for crop, weed, and residue management for winter wheat sub-plots from September 2012 to August 2016.

Lentils (cv. Richlea) were seeded in both organic and conventional production systems. Grazed-organic and tilled-organic sub-plots were seeded on 2 May 2013, 15 May 2014, 24 April 2015, and 21 April 2016 at the rate of 125 kg ha⁻¹ with 15 cm row spacing by using a Fabro (Swift Current, SK, Canada) disk seeder. The seeds were inoculated with N-Dure organic inoculant at the rate of 6.52×10^5 cfu *Rhizobium leguminosarum* bv. *viceae* per kg seed in 2015 and 2016 but not in 2013 and 2014. Chemical no-till sub-plots were seeded on 2 May 2013, 2 May 2014, 14 April 2015, and 19 April 2016 at the rate of at the rate of 63 kg ha⁻¹ in 2013 and 2014, but 67 kg ha⁻¹ seeding rate was used in 2015 and 2016 with 30 cm row spacing. Seeds were treated with Apron Maxx (mefenoxam and fludioxonil) at the rate of 0.06 gm a.i. kg⁻¹ of seed in all years. Supplementary Table 4.S2 summarizes the agronomic practices used for crop, weed, and residue management practices for lentil sub-plots from May 2013 to August 2016.

Disease Assessment

During each growing season, a total of 25 plants in each split-plot were collected from five sampling spots by using a stratified random sampling following a “W” pattern.

Five winter wheat plants were randomly selected from each sampling spot at three growth stages: Feekes 4-5 (tillering), Feekes 10 (flowering), and Feekes 11.4 (maturity). Five lentil plants were also sampled by using a similar stratified random sampling from each sampling spot three times at vegetative growth stage (V6), full bloom stage (R2), and (R6) full seed stage (Erskine et al. 1990).

During each sampling period, whole plants with roots were collected and disease incidence was recorded as the proportion of diseased plants within sampling unit. Disease severity was estimated as the overall percentage of disease area of a plant affected by one or more pathogens, recorded on the basis of symptoms in roots, crown, and leaves (Smiley et al. 2005; Cox et al. 2010; Fernandez et al. 2009). Pathogen identification was determined at the MSU Schutter Diagnostic Lab, Montana State University using a moist chamber and/or plating on artificial media, as appropriate for the fungal species. Fungal pathogens were identified with the resources available in compendium of wheat diseases and pest (Bockus et al. 2010) as well as compendium for chickpea and lentil disease and pests (Chen et al. 2011).

Statistical Analysis

To assess the relationship between disease incidence and cover crop termination methods on winter wheat or lentils, we fitted a generalized linear mixed-effects model with a binomial distribution for each crop. Winter wheat (Y3), Winter wheat (Y5), and lentil were analysed separately. All analyses were performed in R version 3.0.1 (R-Development Core Team, 2014) using the lme4 package (Bates et al. 2015). Disease

incidence was measured as the number of plants infected divided by the total number of plants sampled within each split-plot. The data of zero infection out of total samples (probability = 0) were transformed, as the logit function is undefined with zero values (Ramsey and Schafer 2002). During the transformation, sample size of data that had probability 0 was doubled and added one success (Agresti and Caffo 2000; Agresti and Coul 1998; Warton and Hui 2011). Results are presented as the untransformed probability of disease incidence for clarity. The relationship of cover crop termination methods in cropping system and disease incidence in each crop was tested by competing richness models. Cropping system, growth stage, and year (trial) were modeled as fixed effects, the unique identity of each split-plot (block and split-plot) was added as a random effect to account for repeated measures of the growth stage of the split-plot, and block was also added as random effects to the model to account blocking variability. Trial was modeled as a fixed effect to test for consistency of results between years. The best-fit model was selected with the lowest Akaike's information criterion (AIC) values and used for interpretation (Bolker et al. 2009, Burnham and Anderson 2002; Garrett et al. 2004).

A linear mixed model was used to assess the relationship between disease severity and cover crop termination methods on each crop. Cropping system, growth stage, and year (trial) were modeled as fixed effects, the unique identity of each split-plot (block and split-plot) was added as a random effect to account for repeated measures of the growth stage of the split-plot, and block was also added as random effects to the model to account blocking variability. Homogeneity of variance and normality were visually evaluated by using residual and Q-Q plots. Severity data in the response phase were natural log-transformed to improve the normality but untransformed data are presented in

the results for clarity. Model selection was described above. General linear hypothesis test (glht) with Tukey multiple comparisons in MULTCOMP package were used to compare differences among cropping system (Hothorn et al. 2015). Packages ‘sciplot’ (Morales 2012) and ‘ggplot2’ (Wickham 2009) were used to build graphs.

In 2015, grazed organic winter wheat (Y5) split-plots were not sampled due to repeated grazing. Therefore, 2015 data compared only tilled organic and chemical no till plots. In lentil plots, disease incidence was observed only on vegetative stage in 2013 and 2014. There was no disease infection in 2015 in all stages. In 2016, disease incidence was found only in full seed stage. Therefore, data of 2013, 2014, and 2016 were analyzed only on the treatment level regardless of the growth stages of the lentils.

Results

The common diseases and pathogens infecting winter wheat (both Y3 and Y5) during the study period were Pythium root rot (*Pythium* sp), Rhizoctonia root rot (*Rhizoctonia solani*), common root rot (*Cochliobolus sativus*), tan spot (*Pyrenophora tritici-repentis*), crown rot (*Fusarium* spp.), sharp eye spot (*Rhizoctonia cerealis*), powdery mildew (*Blumeria graminis*), and stripe rust (*Puccinia striatiifolium*). Supplementary Table from 4.S3 to 4.S8 summarizes the types of pathogens their frequency of incidence and relative percentage infecting on different growth stages of winter wheat (Y3 and Y5) in grazed organic, tilled organic and chemical no till system. *Pythium* sp, and *Rhizoctonia solani* in vegetative stage, and *Alternaria* spp. in full seed stage were the only pathogens recorded from lentils.

Disease Incidence and Severity in Winter Wheat (Y3)

Winter wheat (Y3) is the phase which followed the cover crop termination treatments (chemical, tillage and grazing) applied across the system. Disease incidence associated with cropping systems depended on growth stages of winter wheat and year (system \times stage \times year, $\chi^2 = 124.9$, $df = 12$, $P < 0.01$). In 2013, at tillering, flowering, and matured stages, disease incidence was similar between chemical no till, grazed and tilled organic system (Fig. 4.1A) ($P > 0.1$ in all contrasts). In 2014, organic system had higher disease incidence than chemical no till (Fig 4.1). There was no difference in disease incidence between cropping system at tillering and flowering stage in 2014(Fig. 4.1B). At 2014 matured stage, chemical no till ($29 \pm 7\%$) had lower disease incidence than grazed organic ($90 \pm 7\%$, $P < 0.01$) and tilled organic system ($72 \pm 12\%$, $P < 0.01$), but grazed and tilled organic had similar disease incidence ($P = 0.18$).

In 2015, chemical no till and tilled organic had higher disease incidence than grazed organic (Fig. 4.1). At the tillering stage in 2015, grazed organic ($24 \pm 12\%$) had a lower disease incidence than chemical no till ($84 \pm 16\%$, $P < 0.01$) and tilled organic system ($96 \pm 4\%$, $P < 0.01$), but chemical no till and tilled organic had similar disease incidence ($P = 0.89$) (Fig. 4.1C). In 2015, at the flowering stage, grazed organic ($24 \pm 11\%$) had a lower disease incidence than chemical no till ($96 \pm 2\%$, $P < 0.01$), and tilled organic system ($66 \pm 7\%$, $P = 0.03$), and chemical no till had a higher incidence than tilled organic ($P < 0.01$). Similarly, at the 2015 matured stage, grazed organic ($24 \pm 4\%$) had lower disease incidence than chemical no till ($93 \pm 4\%$, $P < 0.01$) and tilled organic ($96 \pm 3\%$, $P < 0.01$), but chemical no till and tilled organic had similar disease incidence

($P = 0.99$). In 2016, conventional no till had higher higher disease incidence than both organic system. At the tillering and flowering stages in 2016, disease incidence was similar among chemical no till, grazed organic, and tilled organic system (Fig 4.1D) ($P > 0.1$ in all contrasts). However, at matured stage, chemical no till ($88 \pm 7 \%$) had higher disease incidence than grazed organic ($41 \pm 6 \%$, $P < 0.01$) and tilled organic ($49 \pm 13 \%$, $P = 0.01$), and there was similar disease incidence between grazed organic and tilled organic system ($P = 0.99$).

Disease severity associated with cropping system depended on growth stages of winter wheat and year (system \times stage \times year, $\chi^2 = 29.82$, $df = 12$, $P < 0.01$). Disease severity was similar in 2013, 2014, 2016 except in 2015 between systems and growth stages of winter wheat (Fig. 4.2). During 2013, disease severity ranged between 1.6 % and 6.0 % and was similar between growth stages and between grazed organic, tilled organic and chemical no till system ($P = 0.99$ for all contrasts) (Fig. 4.2A). In 2014, disease severity was also low ranged between 1.0 % and 6.7 % and similar across management systems within growth stages of winter wheat ($P = 0.99$, in all contrasts) (Fig. 4.2B). In 2015 tillering stage, tilled organic system ($8.5 \pm 1.2 \%$) had higher severity than grazed organic ($1.4 \pm 1.5 \%$, $P < 0.01$), but tilled organic had similar severity than chem no till ($P = 0.99$) (Fig. 4.2C). At the 2015 flowering and matured stage, disease severity ranged between 1.3 % and 5.0 % and similar between systems ($P = 0.99$, in all contrasts). In 2016, disease severity ranged between 1.0 % and 2.2 % and was similar between growth stages and between grazed organic, tilled organic and chemical no till system ($P = 0.99$ for all contrasts) (Fig. 4.2D).

Disease Incidence and Severity in Winter Wheat (Y5)

Disease incidence among cropping system depended on growth stages of winter wheat and year (system \times stage \times year, $\chi^2 = 15.7$, df = 8, $P = 0.04$). In 2013 and 2014, at tillering, flowering, and mature stages, disease incidence was similar between chemical no till, grazed and tilled organic within growth stages (Fig. 4.3A and B) ($P > 0.1$ in all contrasts). In 2015, at the tillering stage chemical no till ($97 \pm 3\%$) had a higher disease incidence than tilled organic system ($76 \pm 6\%$, $P < 0.01$), but at flowering and mature stages both system had similar disease incidence ($P > 0.98$) (Fig. 4.3C). In 2016, disease incidence was similar among chemical no till, grazed organic, and tilled organic system within all three growth stages of winter wheat (Fig 4.3D) ($P > 0.1$ for all contrasts).

Disease severity was not different between cropping system, growth stages of winter wheat and year (system \times stage \times year, $\chi^2 = 13.2$, df = 8, $P = 0.10$). Disease severity was similar between systems ($\chi^2 = 3.6$, df = 2, $P = 0.15$) and it was similar across years (system \times year, $\chi^2 = 1.2$, df = 4, $P = 0.87$). Disease severity was ranged low and ranged between 1.6 % and 6.8 % (Fig.4.4).

Disease Incidence and Severity on Lentils

Disease incidence differed among grazed organic, tilled organic, and chemical no till system and year (system \times year, $\chi^2 = 15.2$, df = 4, $P < 0.01$). In 2013 there was similar probability of disease incidence between chemical no till ($22 \pm 5\%$) and grazed organic ($12 \pm 8\%$, $P = 0.43$); chemical no till and tilled organic ($20 \pm 4\%$, $P = 0.99$), as well grazed and till organic ($P = 0.63$) (Fig. 4.5). However, in 2014, tilled organic ($3 \pm 3\%$)

had lower probability of disease incidence than grazed organic ($30 \pm 12\%$, $P < 0.01$) but not with chemical no till ($20 \pm 6\%$, $P = 0.11$); chemical no till and grazed organic had similar disease incidence ($P = 0.98$). In 2016, disease incidence ranged from 9% to 21% and similar across system ($P > 0.82$ in all contrast).

Disease severity associated with cropping system did not depend on years (system \times year, $\chi^2 = 5.3$, $df = 4$, $P = 0.25$). Disease severity was similar between grazed organic, chemical no till and tilled organic system ($P > 0.05$) and between years ($P = 0.08$). Disease severity was low and ranged between 1.7% and 3.4% (Fig. 4.6).

Discussion

The disease incidence in subsequent winter wheat (Y3) following cover crop varied from years and at growth stages of winter wheat. This variability is possibly due to the types of pathogen prevalent at specific growth stages of winter wheat, plant diversity between systems and year to year variability in environmental factors. For example, there was low disease incidence in chemical no till than both organic systems in 2014. In that year, both organic systems were highly infected with common root rot, a soil-borne disease compared to chemical no till. In 2015, grazed organic system had lower disease incidence at all stages of winter wheat compared to tilled organic and chemical no till; strip rust and powdery mildew were most frequent disease in this year. Lower disease incidence of these foliar pathogens in grazed organic system might be due to the highly diversified grazed organic plots and the transmission of host specific pathogen like rust and powdery mildew decreases when diversity increase according to the dilution effect of

host-diversity hypothesis. This result indicates that widely dispersed diseases like rust would be less likely to reflect management treatments than dispersal limited diseases such as soil-borne pathogens. In other study comparing organic and conventional production, beneficial microbial activity has higher in organic farming (vanBruggen and Termorshuizen 2003). We had no direct measure of micro-organisms across organic and conventional system. A long term study evaluating the diversity of microorganism can help to differentiate the effect of disease suppression by beneficial microorganism to the pathogens. In a long-term comparative study of wheat diseases such as sharp eye spot (caused by *Rhizoctonia solani*) and foot rot (caused by *Fusarium* spp.) indicated these stubble-borne crown rot were less severe in organic farming than in the conventional farming (Piorr and Hindorf 1986; Daamen et al. 1989; Tamis and van den Brink 1998). However, damping off caused by *Cochliobolus sativus* had higher incidence in organic farms than conventional ones (Hannukkala and Tapio 1990).

Disease incidence in winter wheat (Y5) and lentils during transition to organic in 2013 and 2014 as well as established organic year 2016 was similar across the grazed organic, tilled organic and chemical no till system except at tillering stage in 2015 in winter wheat (Y5) and 2014 in lentil. The lack of differences in disease incidence among cropping systems possibly due to the presence of similar pathogen in different growth stages of winter wheat and cover crop termination methods had low or no impact after two to three years (supplementary table 4.S5-8). Past research for example, Bailey et al. (2001) found that disease caused *Cochliobolus sativus* decreased and *Fusarium* spp. increased under reduced tillage compared to conventional tillage wheat fields but there was no difference in leaf spot diseases between tillage practices. In another study, Bailey

et al. (2000) found that the foliar disease tan spot caused by *Pyrenophora tritici-repentis* was more prevalent in no-till wheat fields than conventional tillage, but tillage practices had no measurable impact on lentil diseases in the same study. The possible reason of similar disease incidence in this study was due to overall disease incidence caused by several pathogens. This study did not focus on one type of pathogen; the disease incidence recorded was the disease symptoms, which were possibly caused by more than one pathogen.

The variability of disease incidence across growth stages of winter wheat might be due to the impact of factors such as disease prevalence in the surrounding, weather variables, and micro-climatic conditions including changes in soil and canopy moisture and temperature across fields and between years (Campbell and Neher 1994). While evaluating the impact of these variables in wheat disease incidence is beyond the scope of this study, Bailey et al. (2000, 2001) found that 59 % to 75 % of the variation in severity of wheat diseases was accounted for annual environmental factors and not by agronomic practices including rotation and tillage. Future research could analyze the impact of weather variables, soil chemical conditions, and micro-organisms across the tilled organic, grazed organic and conventional no-till systems help to explain the impact of cover crop termination methods in depth.

Disease severity is an important component of disease assessment as it can be directly impact crop yield (Campbell and Neher 1994). Although, we observed high disease incidence, disease severity was low throughout the study period in winter wheat and lentils. There was no difference in disease severity in the transition to organic period and established organic year between the grazed organic, tilled organic and chemical no

till system. There was no evidence that disease severity impacted on yields since yields were similar in winter wheat (WW3) from 2013 and 2014 (Johnson (2015)). The result of similar severity between systems is supported by previous researches. Smiley et al. (1996) found that severity of *Rhizoctonia* root rot did not differ between tilled and no-till plots after 25 years of a wheat-pea rotation. Likewise, Schroeder and Paulitz (2006) found similar severity of *Pythium* root rot between tilled and direct seeded plots. Bailey et al. (2000, 2001) also recorded similar overall disease severity of caused by *Cochliobolus sativus* and *Fusarium* spp. and wheat yield between conventional tilled and no-till plots. Cover crops have been shown to increase the population of beneficial organisms in soil and reduce disease severity (Schutter and Dick 2002), Soil organic matter is important to maintain microbial suppression of soilborne diseases (Feng et al. 2003; Larkin and Honeycut 2006; van Elsas et al. 2002). The impact of management practice decisions takes long-term comparative studies and can be highly variable.

Overall this study shows that with the integration of cover crops and targeted sheep grazing in to a cropping system, a grower can expand their organic production without fear of increased disease risk during transition to organic period. This study also indicates sheep grazing can be an alternative to tillage in transition to organic and newly certified organic land. Lease grazing is an alternative for ranches and for both organic and conventional growers to reduce the shortcomings of tillage and herbicide application for cover crop termination in dryland farming (Thiessen Mortens and Entz 2011).

Table 4.1. Average monthly temperature and total monthly and annual precipitation from 2012 to 2016 near Bozeman, Montana

Months	Temperature (°C)						Precipitation (mm)					
	2012	2013	2014	2015	2016	LTA*	2012	2013	2014	2015	2016	LTA*
January	-1.2	-4.4	-2.1	-2.3	-3.7	-5.5	9.1	18.3	17.5	13.5	20.6	21.8
February	-2.5	-2.2	-8.1	1.3	1.5	-3.6	13.5	9.7	35.5	14	9.9	18.5
March	5.3	1.8	0.4	5.5	4.1	0.2	42.9	17.5	87.6	15.5	53.1	34.5
April	8.5	4.6	5.9	6.7	8.3	5.7	83.8	23.9	56.4	54.4	52.8	48
May	9.8	11.2	10.9	10.4	10.2	10.5	61.0	109.5	47.8	80	54.9	73.4
June	15.9	15.8	13.5	18.5	17.8	14.7	39.1	87.6	95.3	14.7	22.1	73.4
July	21.3	20.5	20.7	19.1	18.9	19.1	28.7	18	15.5	64.5	31	34.3
August	19.9	19.8	18.2	19.1		18.3	14.7	18.8	72.1	31		31.8
September	15.5	14.6	14.1	15.0		13.0	5.3	76.2	40.9	40.9		43.4
October	7.4	5.3	10.8	10.4		7.4	20.1	25.4	21.8	44.2		38.9
November	2.6	1.1	-1.7	-1.5		0.2	28.7	23.6	41.4	38.1		28.4
December	-3.7	-6.1	-2.2	-3.9		-4.4	34.5	31.8	26.7	39.1		22.6
Annual total	-	-	-	-		-	381.5	460.2	558.3	449.8		469.1

* Long-term average, 124 years (1892-2016) mean temperature (°C), and precipitation (mm) for Bozeman, Montana located approximately 5 km southwest from the experiment site, ID USC00241044 (Source: Western Regional Climate Center, Reno, NV, USA).

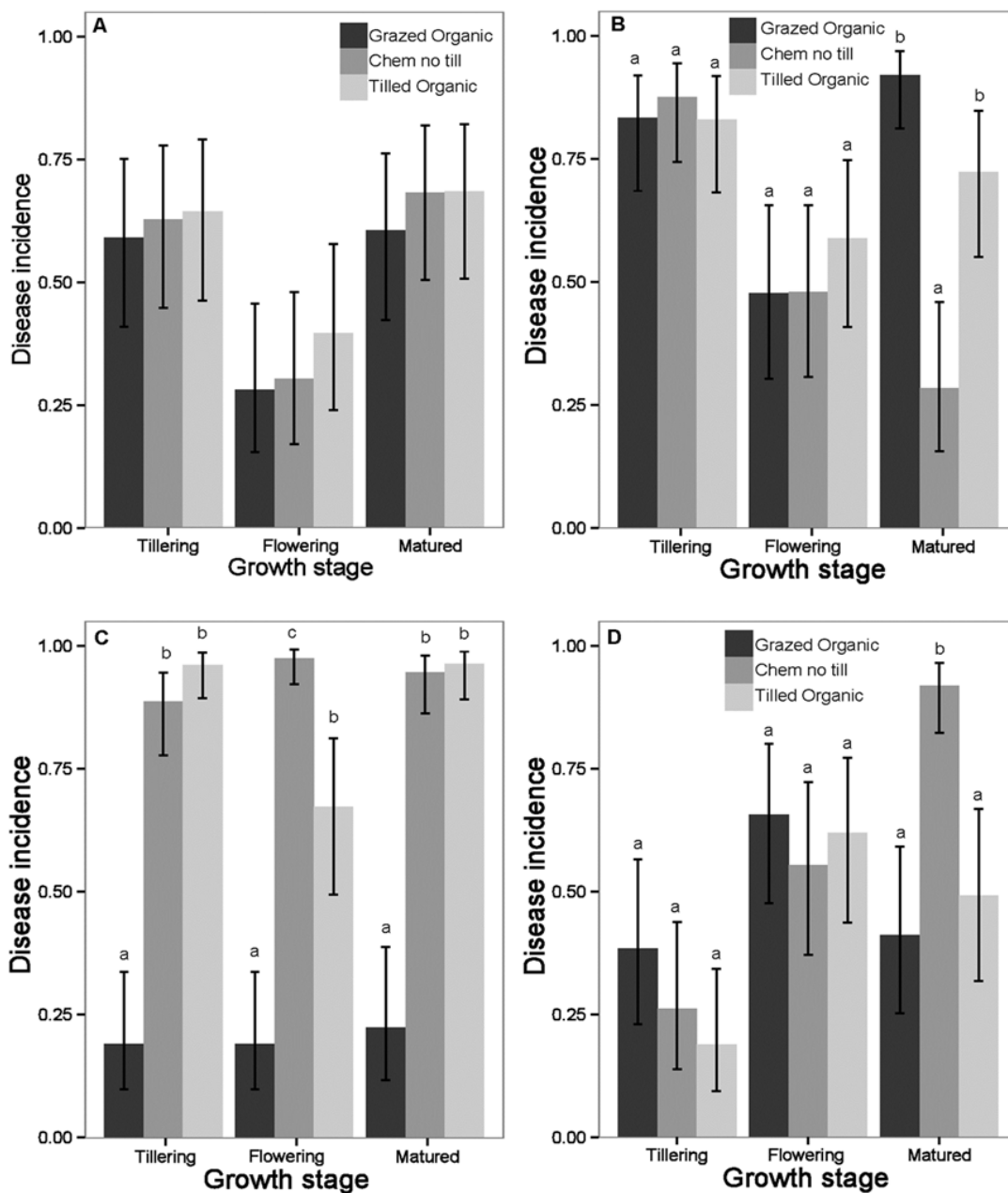


Fig.4.1. Average disease incidence on winter wheat (Y3) across chemical no till, grazed and tilled organic system at three growth stages Feekes 4-5 (tillering), Feekes 10 (flowering) and Feekes 11.4 (maturity) of winter wheat for **A**, 2013; **B**, 2014; **C**, 2015; **D**, 2016. Disease incidence was estimated as the proportion of diseased plants within sampling unit. Different letters within year and growth stage indicate significant differences between cropping system ($P < 0.05$). Error bars are 95% confidence interval.

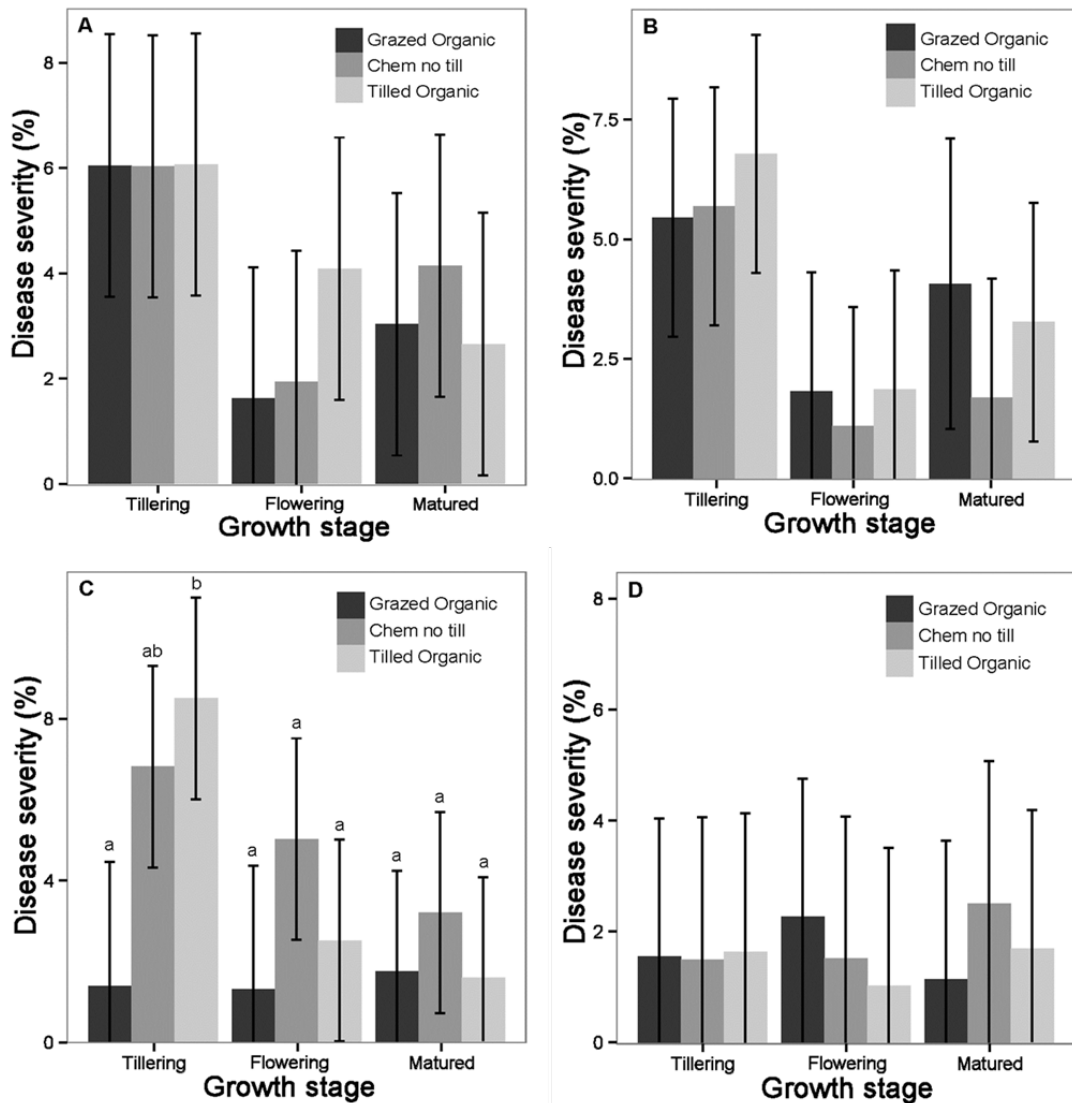


Fig.4.2. Average disease severity on winter wheat (Y3) across chemical no till, grazed and tilled organic system at three growth stages Feekes 4-5 (tillering), Feekes 10 (flowering) and Feekes 11.4 (maturity) of winter wheat for **A**, 2013; **B**, 2014; **C**, 2015; **D**, 2016. Disease severity was estimated as the percentage of plant area that was affected by diseases in a plant. Different letters within growth stage indicate significant differences between cropping system ($P < 0.05$). Error bars are 95% confidence interval.

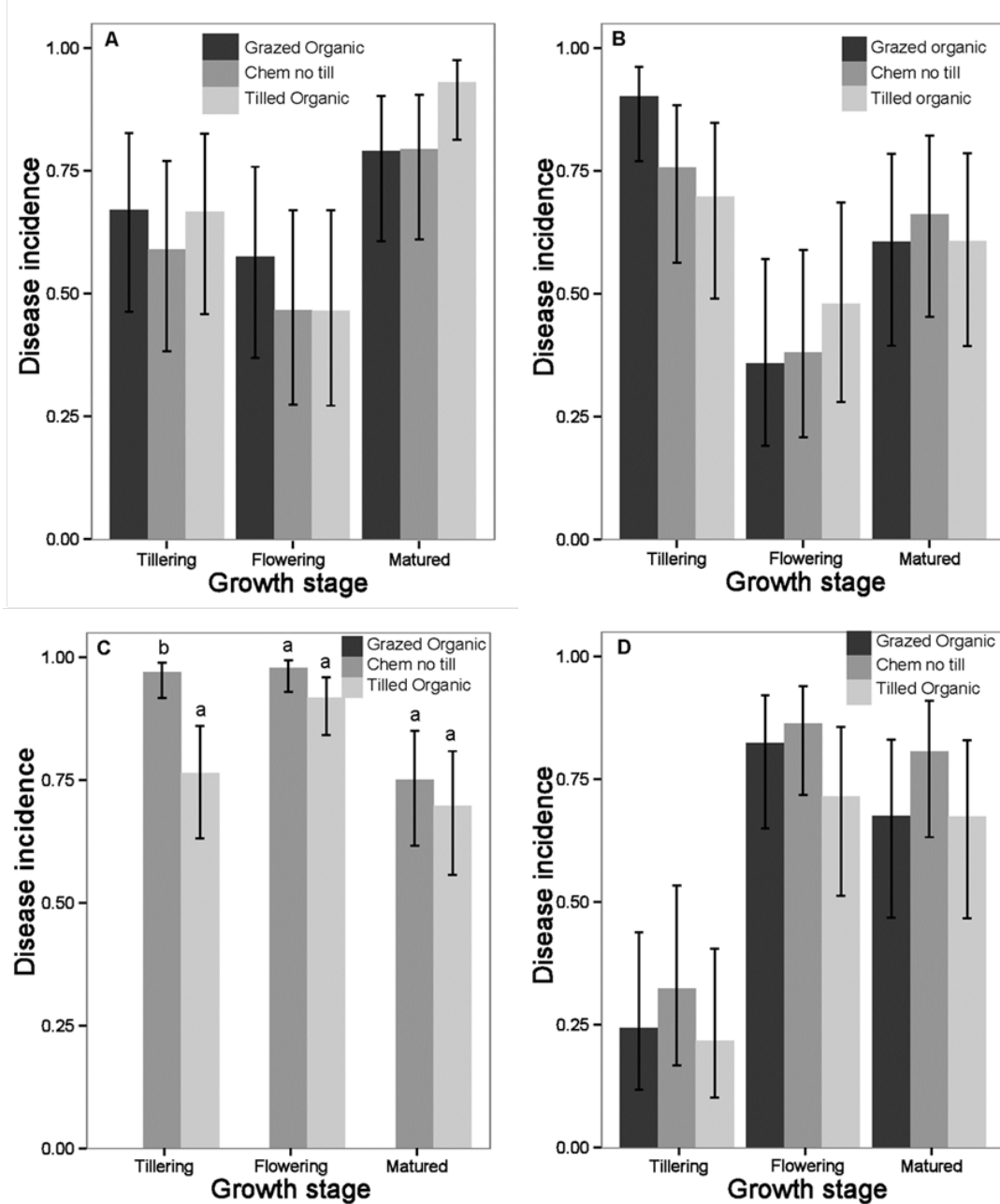


Fig.4.3. Average disease incidence on winter wheat (Y5) across chemical no till, grazed and tilled organic system at three growth stages Feekes 4-5 (tillering), Feekes 10 (flowering) and Feekes 11.4 (matured) of winter wheat for **A**, 2013; **B**, 2014; **C**, 2015; **D**, 2016. Disease incidence was estimated as the proportion of diseased plants within sampling unit. Different letters within growth stage indicate significant differences between cropping system ($P < 0.05$). Error bars are 95% confidence interval.

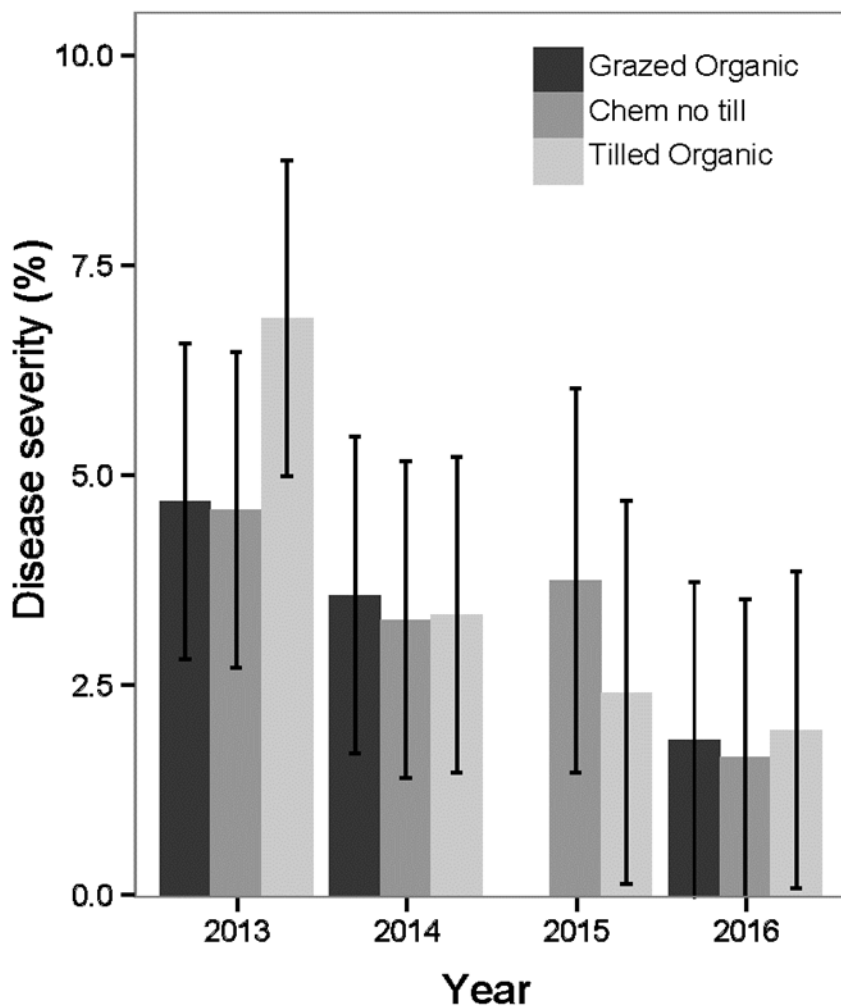


Fig.4.4. Average disease severity on winter wheat (Y5) across chemical no till, grazed and tilled organic. Plant samples were taken at Feekes 4-5 (tillering), Feekes 10 (flowering) and Feekes 11.4 (maturity) of winter wheat. Disease severity was estimated as the percentage of plant area that was affected by diseases in a plant. Error bars are 95% confidence interval.

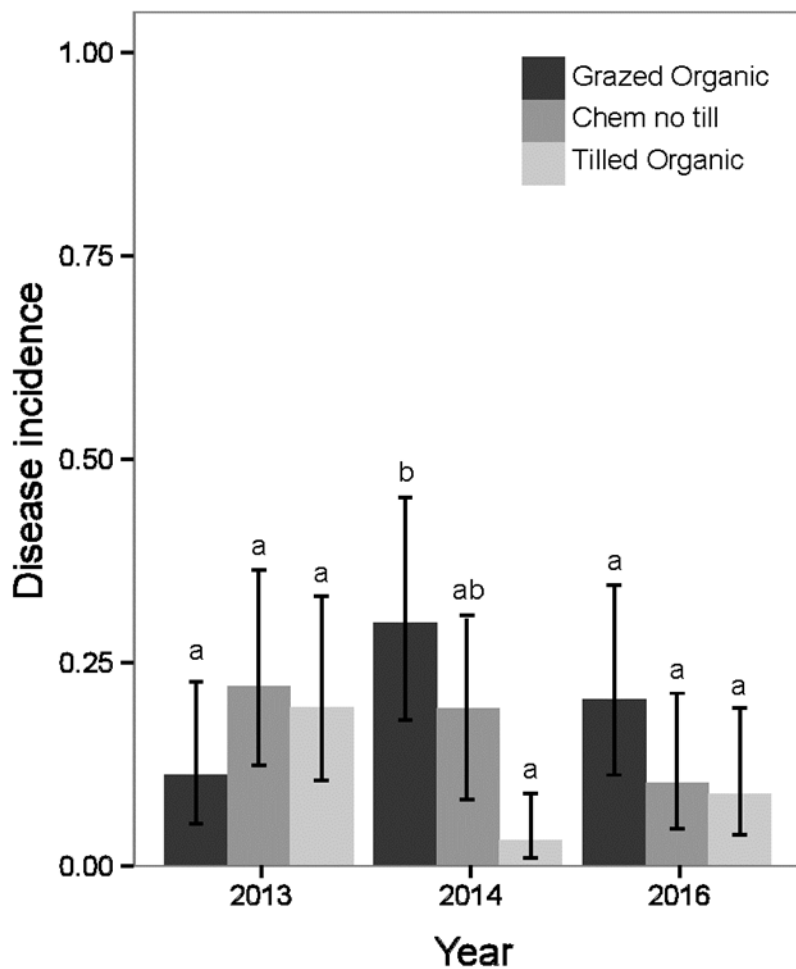


Fig.4.5. Average disease incidence on lentils (cv. Richlea) across chemical no till, grazed and tilled organic system. There was no disease incidence on 2015. Disease incidence was estimated as the proportion of diseased plants within sampling unit. Different letters within year indicate significant differences between cropping system ($P < 0.05$). Error bars are 95% confidence interval.

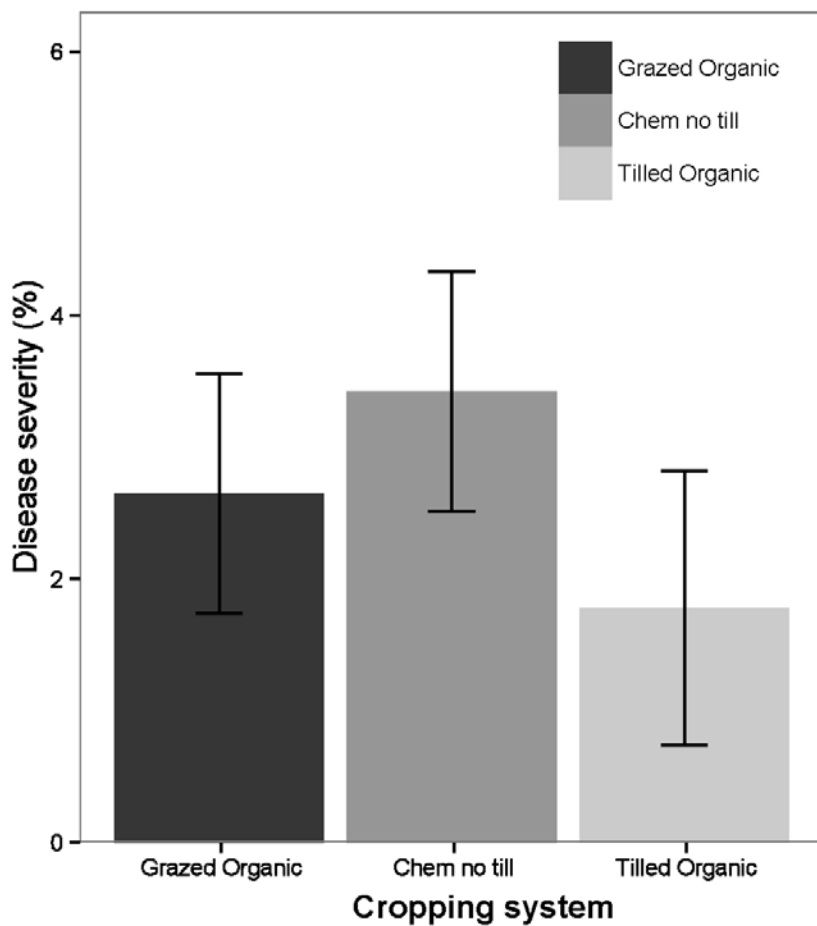


Fig.4.6. Average disease severity on lentils (cv. Richlea) across grazed organic, chemical no till, and tilled organic system. Disease severity was estimated as the percentage of plant area that was affected by diseases in a plant. Error bars are 95% confidence interval.

Supplemented Table 4.S1: Crop management in tilled-organic, grazed-organic and chemical no-till winter wheat plots at Fort Ellis, Montana

year	tilled-Organic	grazed- organic	chemical no-till
Sep. 2012 to Oct. 2013	Residue incorporated with offset disk on 16 Sep 2013	Grazed residue with 100 lambs/ha from 1 Oct to 25 Nov 2013 for feedlot	
	chisel plowed on 23 Sep 2013		Post-emergent dicamba (105 gm ae/ha) mixed with 2,4-D (482 gm ai/ha) on 14 may 2013 270-370 kg/ha of 46-0-0 urea applied on 15 may 2013 using Vlamar 30cm spreader Sprayed with glyphosate (112 gm ai/ha) on 16 Sep 2013 using 64 cm shielded boom sprayer
Sep. 2013 to Oct. 2014	Rotary hoed stubble on 18 August 2014	Rotary hoed stubble on 19 August 2014	130 kg/ha of 46-0-0 urea applied on 23 Sep 2013 on lentil stubble plots only
	Tilled with chisel plot on 29 August 2014	Tilled with Chisel plow on 4 Sep 2014	Post-emergent pinoxaden (60 g ae/ha), fluroxypyr (105 g ae/ha), bromoxynil (263 g ai/ha), and MCPA (263 g ai/ha) on 2 May 2014
		Grazed plots at 100 sheep/ha on 5 Sep 2014	Rotary hoed stubble 19 Aug 2014 Sprayed glyphosate (1120 g ai/ha) 16 on 1 Sep 2014
Sep. 2014 to Oct. 2015	Rotary hoed stubble on 17 Sep 2014	Grazed residue with 100 lambs/ha from 22 Sep to 4 Nov 2014 for feedlot	110 kg/ha of 46-0-0 urea applied on 4 may 2015 using Vlamar 30cm spreader
	Tilled with chisel plot on 17 Sep 2014	Three winter wheat plots grazed with 234 rams/ha from 29 April to 3 May 2015	Sprayed Huskie® (228 gm ai/ha) mixed with 2,4-D (619.2 gam ai/ha) and AMS (1120.85 gm ai/ ha) on 22 April 2015
		Three winter wheat plots grazed with 250 rams/ha from 12 April to 3 May 2015	
		Grazed with at 100 ram per ha from 20 July to 6 August 2015 on Y5 WW plots	
		Grazed with at 100 ram per ha from 1 Sep to 6 August 2015 on Y5 WW plots	
Sep. 2015 to Oct. 2016			Sprayed glyphosate (818.8 g ai/ha) with 0.5 % v/v Hel-fire adjuvant at 93.5 l/ha water volume on 12 Oct 2015 sprayed GoldSky (145.37 gm ai / ha with 0.5 % v/v R11 at 93.5 l/ha On 7 April 2016 245.5 kg/ha of 46-0-0 urea applied on 11 April 2015 using Vlamar 30cm spreader

Supplemented Table 4.S2: Crop management in tilled-organic, grazed-organic and chemical no-till lentil plots at Fort Ellis, Montana.

year	tilled-Organic	grazed- organic	chemical no-till
Sep-12	-----Cultivated 19 April 2013 with a spring tine cultivator with shovels and a trailing basket-----		
to Oct 2013	-----Cultivated 25 April 2013 with chisel plow with 40-cm shovels and trailing spring tine harrows-----		
	Rolled plots 1 May 2013	Grazed weeds and residue on 28 Aug	Sprayed with glyphosate (1120 g ai/ha)
	Tilled 8 Aug 2013 with chisel plow	2013 at rate of 167 lambs/ha	with AMS on 16 Sep 2013
	Tilled with chisel plow 28 August 2013		
	Tilled with spring-tine cultivator with		
	18-cm shovels and trailing roller		
	baskets on 8 Oct 2013		
Sep-13	-----Tilled with chisel plough on 2 May 2014-----		
to Oct 2014	-----Spring-tined cultivated on 14 May 2014-----		
	Tilled with chisel plough on 10 Oct 2013		
	Tilled with chisel plough with 40-cm	Grazed with at 100 rambouillets per ha	Rolled plots on 23 May 2014
	shovels on 18 August 2014	from 15-18 August 2014	Sprayed weeds with glyphosate (1120 h
	Spring-tined cultivated on 29 August 2014	Grazed at 208 lambs/ha from 16-22 Sep	ai/ha) 16 Sep 2014
		2014	
Sep-14	-----Tilled with chisel plough on 14 April 2015-----		
to Oct 2015	-----Spring-tined cultivated on 14 April 2015-----		
	Tilled with chisel plough on 4 August 2013		sprayed with Huskie (228 gm ai/ha) mixed
			with 2, 4 D (619.2 gm ai/ ha) with
			AMS (1120.85 gm/ha) on 22 April 2015
			Sprayed Clethodim (221.92 gm ai/ha) with
			1% v/v crop oil concentration (9.6 gm/lit)
			on 2 June 2015
			Sprayed with glyphosate (1086.1 g ai/ha)
			with AMS (18 gm/ l) at 93.5 l/ha water volume
			on 10 and 21 August 2015
Sep-15	-----Tilled with chisel plough on 9 April 2016-----		
to Oct 2016			Sprayed with glyphosate (818.8 g ai/ha)
			with 0.5 % v/v Hel-fire adjuvant at 112.2 l/ha
			tank mixed with 70 gm/ha Sharen and 1%
			v/v MSO at 93.5 l/ha spray volume
			on 18 April 2016
			Sprayed Assure II (57.72 gm ai/ha) with
			1% v/v crop oil concentration and 0.25% R11
			on 26 May 2016

Supplemented Table 4.S3: Diseases of winter wheat (Y3) and their frequency and percentages recorded at tillering stage of winter wheat in chemical no-till, grazed-organic and tilled-organic plots at Fort Ellis, from 2013 to 2016

Treatment	year	# infected	RHIZ		PYTH		RHIZ+PYTH		TS		PYTH+TS		RHIZ+PYTH+TS		CRR		PM		PM+PYTH		PM+RHIZ		RHIZ+TS		
			f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f
Chem no till	2013	47	7	14.9	25	53.2	15	31.9																	
Chem no till	2014	67	3	4.5	59	88.1	5	7.5																	
Chem no till	2015	63			43	68.3	4	6.3	1	1.6	11	17.5	4	6.3											
Chem no till	2016	19	6	31.6	6	31.6	7	36.8																	
Grazed Organic	2013	44	8	18.2	25	56.8	11	25.0																	
Grazed Organic	2014	62	3	4.8	39	62.9	20	32.3																	
Grazed Organic	2015	17	6	35.3	4	23.5		0.0							7	41.2									
Grazed Organic	2016	29	10	34.5	14	48.3		0.0			1	3.4	2	6.9									2	6.9	
Tilled Organic	2013	48	2	4.2	25	52.1	21	43.8																	
Tilled Organic	2014	64	6	9.4	58	90.6	0	0.0																	
Tilled Organic	2015	72			13	18.1	1	1.4			12	16.7	8	11.1			6	8.3	25	34.7	7	9.7			
Tilled Organic	2016	15	9	60.0	2	13.3	2	13.3															2	13.3	

Where, RHIZ = Rhizoctonia root rot, PYTH = Pythium root rot, TS = Tan spot, CRR = Common Root rot, PM= Powdery mildew

Supplemented Table 4.S4: Diseases of winter wheat (Y3) and their frequency and percentages recorded at flowering stage of winter wheat in chemical no-till, grazed-organic and tilled-organic plots at Fort Ellis, from 2013 to 2016

Cropping system	year	# infected	TS		CRR		ES		FUS		TS+CRR		PM		Rust		ES+Fus		Rust+ES		CRR+RUST		TS+Rust		TS+ES	
			f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%
Chem no till	2013	23	17	73.9	5	21.7	1	4.3																		
Chem no till	2014	34	30	88.2	3	8.8					1	2.9														
Chem no till	2015	72	36	50.0	2	2.8					3	4.2	31	43.1												
Chem no till	2016	39											8	20.5	31	79.5										
Graed Organic	2013	22	12	54.5	4	18.2	2	9.1	4	18.2																
Graed Organic	2014	32	20	62.5	6	18.8	1	3.1			5	15.6														
Graed Organic	2015	17	10	58.8	7	41.2																				
Graed Organic	2016	49			5	10.2	11	22.4	2	4.1			1	2.0	10	20.4			4	8.2	5	10.2	6	12.2	5	10.2
Tilled Organic	2013	30	5	16.7	20	66.7	4	13.3	1	3.3																
Tilled Organic	2014	38	13	34.2	17	44.7	1	2.6			7	18.4														
Tilled Organic	2015	50			2	4.0							48	96.0												
Tilled Organic	2016	46			6	13.0					2	4.3			25	54.3	1	2.2	1	2.2	3	6.5	8	17.4		

Where, TS = Tan spot, CRR = Common Root rot, ES = sharp Eye spot, FUS = Fusarium root rot, PM= Powdery mildew, Rust = Stripe rust

Supplemented Table 4.S5: Diseases of winter wheat (Y3) and their frequency and percentages recorded at matured stage of winter wheat in chemical no-till, grazed-organic and tilled-organic plots at Fort Ellis, from 2013 to 2016

Cropping system	year	# infected	TS		CRR		ES		FUS		RUST		PM+RUST+FUS+CRR		PM+CRR		FUS+CRR		RUST+CRR		PM+FUS		TS+CRR		PM		FUS+PM		ES+CRR		ES+RUST	
			f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%
Chem no till	2013	51			51	100																										
Chem no till	2014	22			16	72.7	5	22.7	1	4.5																						
Chem no till	2015	70	1	1.4	19	27.1			14	20.0	3	4.3	5	7.1	15	21.4	8	11.4	2	2.9	3	4.3										
Chem no till	2016	66	1	1.5	11	16.7			3	4.5	23	34.8					4	6.1	15	22.7							3	4.5	6	9.1		
Graed Organic	2013	45			43	95.6	2	4.4																								
Graed Organic	2014	45			39	86.7	1	2.2	5	11.1																						
Graed Organic	2015	18			5	27.8			2	11.1	3	16.7	2	11.1	3	16.7		0.0		0.0			2	11.1	1	5.6						
Graed Organic	2016	31			19	61.3	7	22.6			4	12.9													1	3.2						
Tilled Organic	2013	51			49	96.1		0.0	2	3.9																						
Tilled Organic	2014	53			43	81.1	3	5.7	7	13.2																						
Tilled Organic	2015	72			33	45.8	1	1.4	3	4.2		0.0	10	13.9	7	9.7	9	12.5	7	9.7							3	4.2				
Tilled Organic	2016	37			19	51.4	6	16.2			5	13.5	1	2.7								4	10.8	1	2.7			1	2.7			

Where, TS = Tan spot, CRR = Common Root rot, ES = sharp Eye spot, FUS = Fusarium root rot, PM= Powdery mildew, Rust = Stripe rust

Supplemented Table 4.S6: Diseases of winter wheat (Y5) and their frequency and percentages recorded at tillering stage of winter wheat in chemical no-till, grazed-organic and tilled-organic plots at Fort Ellis, from 2013 to 2016

Cropping system	Year	#infected	RHIZ		PYTH		RHIZ+PYTH		CRR		FUS		RHIZ+TS		FUS		TS		TS+PYTH		PM	
			f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%
Chem no till	2013	44	6	13.6	17	38.6	15	34.1	3	6.8	1	2.3	2	4.5		0						
Chem no till	2014	58	6	10.3	42	72.4	10	17.2														
Chem no till	2015	73	2	2.7	21	28.8	36	49.3									3	4.1	11	15.1		
Chem no till	2016	26	10	38.5	9	34.6											7	26.9				
Grazed Organic	2013	51	4	7.8	31	60.8	14	27.5	2	3.9						0						
Grazed Organic	2014	67	6	9.0	51	76.1	10	14.9														
Grazed Organic	2016	19	8	42.1	6	31.6											5	26.3				
Tilled Organic	2013	49	2	4.1	21	42.9	23	46.9	2	4.1					1	2.0						
Tilled Organic	2014	49	1	2.0	46	93.9	2	4.1														
Tilled Organic	2015	57	7	12.3	16	28.1	7	12.3									8	14.0	1	1.8	16	28.1
Tilled Organic	2016	17	2	11.8	4	23.5											11	64.7				

Where, RHIZ = Rhizoctonia root rot, PYTH = Pythium root rot, TS = Tan spot, CRR = Common Root rot, PM= Powdery mildew, FUS = Fusarium root rot

Supplemented Table 4.S7: Diseases of winter wheat (Y5) and their frequency and percentages recorded at flowering stage of winter wheat in chemical no-till, grazed-organic and tilled-organic plots at Fort Ellis, from 2013 to 2016

Cropping system	year	# infected	TS		CRR		ES		FUS		TS+CRR		TS+PM		PM		RUST		ES+FUS		RUST+ES		CRR+RUS		TS+RUST	
			f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%
Chem no till	2013	35	28	80	5	14.3	1	2.9	1	2.9																
Grazed Organic	2013	43	16	37.2	23	53.5			1	2.3	3	6.9														
Tilled Org.	2013	35	14	40.0	16	45.7			5	14.3																
Grazed Organic	2014	28	23	82.1	3	10.7					2	7.1														
Chem min til	2014	23	21	91.3	2	8.7																				
Tilled organic	2014	35	9	25.7	8	22.9	3	8.6			15	42.9														
Chem no till	2015	74	13	17.6	2	2.7							55	74.3	6	8.1										
Tilled Organic	2015	67	22	32.8	2	3.0							36	53.7	7	10.4										
Grazed Organic	2016	61	1	1.6	7	11.5	14	23.0	3	4.9							17	27.9	1	1.6	12	19.7	5	8.2	1	1.6
Chem no till	2016	60			1	1.7	1	1.7									49	81.7			2	3.3	7	11.7		
Tilled organic	2016	53	1	1.9	6	11.3											36	67.9					6	11.3	4	7.5

Where, TS = Tan spot, CRR = Common Root rot, ES = sharp Eye spot, FUS = Fusarium root rot, PM= Powdery mildew, Rust = Stripe rust

Supplemented Table 4.S8: Diseases of winter wheat (Y5) and their frequency and percentages recorded at matured stage of winter wheat in chemical no-till, grazed-organic and tilled-organic plots at Fort Ellis, from 2013 to 2016

Cropping system	year	# infected	CRR		ES		FUS		TS+CRR		TS	RUST		PM+RUST+FUS+CRR		PM+CRR		FUS+CRR		PM+FUS		PM	ES+CRR		ES+RUST		CRR+RUST		TS+RUST			
			f	%	f	%	f	%	f	%		f	%	f	%	f	%	f	%	f	%		f	%	f	%	f	%	f	%	f	%
Chem no till	2013	59	51	86.4			7	11.9	1	1.7																						
Chem no till	2014	45	42	93.3			3	6.7																								
Chem no till	2015	56	32	57.1			2	3.6	1	1.8	3	5.4	2	3.6	7	12.5	5	8.9	1	1.8	3	5.4										
Chem no till	2016	57	26	45.6	9	15.8	2	3.5	3	5.3									0				2	3.5	3	5.3	7	12.3	5	8.8		
Grazed Organic	2013	59	45	76.3			12	20.3																								
Grazed Organic	2014	43	36	83.7			7	16.3																								
Grazed Organic	2016	50	28	56.0	3	6.0	5	10.0				7	14.0						3	6			1	2			3	6				
Tilled Organic	2013	69	56	81.1			13	18.8																								
Tilled Organic	2014	43	37	86.0	3	7.0	3	7.0																								
Tilled Organic	2015	52	35	67.3	1	1.9	11	21.2							1	1.9	2	3.8	1	1.9	1	1.9										
Tilled Organic	2016	50	26	52.0	2	4.0	1	2.0				12	24.0						2	4							7	14				

Where, TS = Tan spot, CRR = Common Root rot, ES = sharp Eye spot, FUS = Fusarium root rot, PM= Powdery mildew, Rust = Stripe rust

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CHAPTER FIVE

SUMMARY OF FINDINGS AND FUTURE RESEARCH

Integrated pest management is at the foundation of sustainable pest management programs aimed at contributing to the global food security (Strange and Scott 2005). Cultural pest management practices are one component of such programs (Noris and Caswell-Chen 2003). These preventive measures can influence pest behavior in ways that reduce crop damage without threatening the sustainability of the agricultural production. In this thesis, I investigated how agronomic practices influence wheat diseases. In my first study, I investigated the influence of alternative hosts on *Wheat streak mosaic virus* (WSMV) incidence and wheat curl mite (WCM, *Aceria tosichella Keifer*) infestation. In the second study, I determined the impact of agronomic practices on WSMV incidence. In the third study, I assessed the impact of cover crop termination methods on disease incidence and severity in wheat (*Triticum aestivum* L.) and lentils (*Lens culinaris* Medik). Overall, these studies provided essential information to develop sustainable disease management strategies.

In my first study (Chapter 2), I investigated the impacts of alternative hosts on WCM populations and how weather influences WCM movement. Since the dynamics of WCM movement to and from wheat fields and alternative hosts in the Northern Great Plains (NGP) is not well documented (Burrows et al. 2009), this study aimed to evaluate the influence of alternative hosts on WCM movement during the fall. To accomplish this study, I used a ‘trap plant’ capture system that mimics volunteer wheat and allowed me to

quantify WCM movement in the fall. As both WSMV and WCM are obligate on living plant tissue, alternative hosts play an important role in maintaining the disease cycle of WSMV. There are two critically important components of disease cycle of WSMV in the fall: first, successful dispersal of WCM from maturing wheat to alternative hosts that allow for WCM and WSMV to survive; and second, reproduction of WCM on those alternate hosts to sustain the population until the new crop emerges and their dispersal to newly emerged crops.

WCM infestation and WSMV infection of trap plants was high at the beginning of the fall which is coincidental with the timing of harvesting of cereal crops and maturation of several predominant grassy weed species. In 2013, alternative hosts had similar WCM infestation on trap plants compared to control during most of the sampled period ($P > 0.05$). In 2014, spring planted *Bromus tectorum* (downy brome, cheatgrass) and volunteer wheat were associated with an increased disease risk for winter wheat due to a high WSMV incidence and WCM abundance in trap plants compared to the control ($P < 0.01$). An observed high abundance of WCM on these hosts indicated that WCM can survive and reproduce successfully on these hosts in the fall. In addition, I observed different nymphs and eggs of mites on plants indicating WCM reproduction.

The impact of fall weather on disease risk was observed across years. In 2013, low WCM infestation and WSMV risk on trap plants was associated with a prolonged cold period. In 2014, temperatures were relatively higher and WCM infestation and WSMV risk increased compared to 2013 (USBR, Great Plains, Agrimet 2015). This trend continued during a prolonged period into mid-October, after which the study was terminated. Similar results were observed in 2015 (data not presented in this thesis).

These results indicate the risk of WCM infestation is higher during warmer falls. Further research that tracks the movement of mites from the alternative hosts to the winter wheat fields by tagging WCM or WSMV as well as the relationship of mite movement with ground or alternative host canopy relative humidity and temperature would provide further data to refine WSMV risk models as related to WCM reproduction and movement. Additionally, future research that assesses the impact of different density of alternative hosts on risk of WSMV infection on wheat would help to make decision of weed control in the field.

The knowledge gained from Chapter 2 could be used in two ways. First, information that warm fall temperatures increase the length of the peak period of WCM infestation and increase the risk of WSMV infection will discourage growers to plant earlier when disease risk is high but they can plant earlier to maximize yieldd (Chapter 3) in a low WSMV risk year. Second, information related to the ability of alternative hosts to support vector populations and persistence of virus, although not completely explored in this study, could help growers identify the high-risk hosts for WCM maintenance and target these species for control. The information obtained in this research is currently being used in collaborative project to develop a forecasting system for WSMV epidemic risk.

In Chapter 3, I investigated how winter wheat variety, planting date, and the timing of nitrogen application could influence WSMV pressure. The experiment had low background disease pressure and unfortunately this experiement failed to test the host-vector-virus interaction. The experimental site in this study had low WSMV incidence and there was almost no infection (mean = 1%) in resistant varieties. In the susceptible

check variety, the early planting date had a higher WSMV incidence (10 %) when compared to the recommended and late planting dates ($P < 0.01$). Early planting coincided with the harvest period for cereal crops and maturation of several alternative host species in the area. I found that yields of winter wheat planted late (3.3 MT ha^{-1}) were lowered than early (5.1 MT ha^{-1} , $P < 0.01$) and recommended (4.5 MT ha^{-1} , $P = 0.02$) planting dates. The difference in yield was likely due to fall establishment of early emerged plants and temperature during grain filling period rather than WSMV incidence. Across resistant varieties, Snowmass and TAM 112 did not show yield penalty due to planting dates. These varieties can be used as WSMV resistant variety in Montana during low background disease pressure. There is agronomic trade-off between yield and planting dates. Grain protein and test weight were higher in the late planting most likely due to the high temperature stress during grain fill. WSMV incidence did not differ due to N application timing in resistant varieties. However, during 2014, WSMV incidence was higher in the early spring (15 %) compared to late spring N application (6 %, $P < 0.01$) in Yellowstone. Likewise, In Pronghorn, early spring N application had WSMV incidence (12%) than fall N application (5%, $P = 0.03$). Although further research with higher WSMV pressure would be more conclusive, the results presented here indicate that early stage of winter wheat N application has the potential to increase disease risk. We found no difference in yield among N application timing and yield ranged between 6.2 MT ha^{-1} and 6.4 MT ha^{-1} , but grain protein was increased from fall to late spring N application. Therefore, I conclude that modifying the timing of N application can reduce the risk of WCM infestation and WSMV incidence with no impact on yield. However, disease

pressure was too low in this study; further study with high disease pressure can be more conclusive.

The information in chapter 3 could be used in several ways by growers. First, although resistant varieties are recommended for WSMV, none are available in Montana (Burrows et al. 2015). These resistant varieties can be a potential option for growers, but further research need to test the efficacy of resistant genes in high WSMV pressure. However, Snowmass and TAM 112 showed that they are well adapted in Montana climate as they have similar yield to locally adapted Yellowstone with low WSM incidence. Secondly, a standard recommendation for WSMV management is delayed planting, which unfortunately I could not measure properly in this study. However, based on the results of Chapter 2, this recommendation is supported when fall temperatures are mild. Thirdly, applying N in the fall is a standard practice in some areas of the state. However, under high WSMV disease risk, I would recommend delaying fertilization until the spring or splitting N applications after symptoms show up and disease prevalence could be assessed. If WSMV symptoms are observed, I would recommend against fertilizing infected plants. This is due to the fact that WCM reproduce more quickly on adequately fertilized wheat plants (Miller et al. 2015) and N application could increase viruliferous mite population as viruliferous WCM reproduce faster than non-viruliferous (Siriwetwivat 2006; Miller et al. 2015). Adding nitrogen to a field infested with viruliferous WCM would only be detrimental to yield and quality with higher input costs and reduced yield due to the disease. Collectively, the findings of this study illustrate that the growers can split the timing and amount of N application to reduce potential risk of WSMV incidence and spread. In addition, this practice could increase N use efficiency

(Sowers et al. 1994) and result in reduced input losses in addition to providing disease management options. Future research that explores the impact of other important nutrient like phosphorus coupled with N on WSMV incidence and estimation of virus titer on different rates of these nutrients would provide more information on potential risk of nutrient application on WSMV epidemics. As with nitrogen, phosphorus has been shown to increase virus replication (Pennazio and Roggero 1997).

In Chapter 4, I studied disease incidence and severity in grazed organic, tilled organic and chemical no till system where sheep grazing, tillage and herbicides were used to terminate cover crop respectively. In this study, the same crop rotation and cover crop was represented in all three cropping system. The common pathogens infecting winter wheat during the study period were *Pythium* sp, *Rhizoctonia solani*, *Cochliobolus sativus*, *Pyrenophora tritici-repentis*, *Fusarium* spp., *Blumeria graminis*, and *Puccinia graminis*. *Pythium* sp, and *Rhizoctonia solani* in vegetative stage, and *Alternaria* sp in full seed stage were the pathogens recorded from lentils.

The disease incidence in subsequent winter wheat (Y3) following cover crop varied from years and at growth stages of winter wheat. This variability is possibly due to the types of pathogen prevalent at specific growth stages of winter wheat, plant diversity between systems and year to year variability in environmental factors. For example, there was low disease incidence in chemical no till than both organic systems in 2014. In that year, both organic systems were highly infected with common root rot, a soil-borne disease compared to chemical no till. In 2015, grazed organic system had lower disease incidence at all stages of winter wheat compared to tilled organic and chemical no till, this might be due to the highly diversified grazed organic plots and the transmission of host

specific pathogen like rust and powdery mildew decreases when diversity increase according to the dilution effect of host-diversity hypothesis. This result indicates that widely dispersed diseases like rust would be less likely to reflect management treatments than dispersal limited diseases such as soil-borne pathogens.

Disease incidence in winter wheat (Y5) and lentils during transition to organic in 2013 and 2014 as well as established organic year 2016 was similar across the grazed organic, tilled organic and chemical no till system and across the growth stages of winter wheat except at tillering stage in 2015 in winter wheat (Y5) and 2014 in lentil. The lack of differences in disease incidence among cropping systems possibly due to the presence of similar pathogen in different growth stages of winter wheat. Disease incidence in winter wheat subsequent to the phase follow the cover crop phase had variable results compared to winter wheat phase five. This indicates that cover crop termination practices impact on disease incidence at specific growth stages of winter wheat.

Disease severity was similar between grazed organic, tilled organic and chemical no till within growth stages of winter wheat in all years ($P > 0.1$ in all contrasts) but different across growth stages in some years ($P < 0.01$ in all contrasts). The possible reason of similar disease incidence in winter wheat phase five (WW5) and disease severity in winter wheat and lentil might be due to overall disease incidence caused by several pathogens in a system level.

Results from Chapter 4 suggests that growers can expect similar disease impacts on winter wheat following a cover crop whether a cover crop is terminated with sheep grazing, tillage or herbicides during the transition to organic phase and early organic year. Additionally, lease grazing is an alternative for ranches and for both organic and

conventional growers to reduce the shortcomings of tillage and herbicide application for cover crop termination in dryland farming (Thiessen Mortens and Entz 2011). Additional information is needed regarding timing and grazing intensity to reduce soil compaction caused by sheep grazing. Soil compaction is known to influence several soilborne plant diseases (Brown 2009; Abawi and Widmer 2000). Additionally, disease impacts may depend upon use of other agronomic practices including sequence of crop rotation and species included in a cover crop. This study consists of single species of cover crop and future research that compares mixtures of cover crops “cocktails” for multiple years may provide more information on the impact of cover crop on disease suppression.

Additionally, we did not assess the types of micro-organisms presence in the soils. Future studies that investigate soil micro-organism diversity of different crop production systems may assist us in determining below ground interaction among beneficial or/and pathogenic microbes.

Understanding factors that affect the ecology of pathogens and vectors as well as the impact of management practices in agroecosystems can provide a basis for ecologically based disease management. Information about the impact of agronomic practices including adjusting planting date, host resistance, nutrient management, use of cover crops, and integration of livestock in the agricultural system can help to better predict and manage disease problems. These practices can influence pest behavior in ways that reduce crop damage without threatening the sustainability of the agricultural production.

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