CHEMICAL CONTROL AND DISEASE RESERVOIR STUDIES OF THE WHEAT CURL MITE (*ACERIA TOSICHELLA* KEIFER), VECTOR TO *WHEAT STREAK MOSAIC VIRUS*

by

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TABLE OF CONTENTS

TABLE OF CONTENTS - CONTINUED

LIST OF TABLES

LIST OF TABLES - CONTINUED

Table Page

ABSTRACT

Wheat streak mosaic virus (WSMV) causes yield loss to wheat (*Triticum aestivum*) in all areas of the world where the crop is grown. No chemical controls for the WSMV vector, the wheat curl mite (WCM, *Aceria tosichella* Keifer), are approved. Control relies primarily on avoiding a 'green-bridge' of living plant material that can host the disease between seasons. This study aimed to 1) identify chemical treatments for WCM control under conventional and organic systems and clarify misconceptions that treatments, such as sulfur, control WCM and 2) analyze the capacity of 20 grassy species to serve as reservoirs of WSMV and WCM. The effects of insecticides with varying modes of action (carbamate, organophosphate, pyrethroid, neonicotinoid, biological control, oil, ovicide, mite growth inhibitor, and soap) on WCM population growth were tested in the greenhouse. Treatment with the active ingredients aldicarb and chlorpyrifos decreased WCM populations compared to untreated controls ($p<0.001$ and $p<0.001$). Field trials were conducted in spring wheat in 2013 and winter wheat in 2013-2014. Similar effects on WSMV spread were not observed in field trials. These trials included ten products consisting of five modes of action: organophosphates, pyrethroid, oil, soap and mite growth inhibitor. Chlorpyrifos was included in the field trials, but no efficacy was seen in 2013 compared to controls under good infection and incidence and infection was low in 2014, therefore we were unable to distinguish any treatment effect. To assess the capacity of 20 grassy species to serve as reservoirs of WSMV and WCM, plants with varying lifespan and origin were grown in the greenhouse and infested with viruliferous WCM. Lifespan had the greatest impact on ability of plants to host WCM ($p=0.011$) and WSMV ($p<0.001$). Annual plant species are more likely to host WCM than perennial grasses, with all species hosting WCM. Native and introduced species tested did not differ in ability to host WCM ($p=0.735$) and WSMV ($p=0.096$). This study provides evidence of potential for use of active ingredient chlorpyrifos in WCM control, and showed that lifespan is an important determinant of WSMV disease reservoir potential of grassy species.

INTRODUCTION

Wheat Streak Mosaic Virus: a Global Picture

Wheat streak mosaic virus (WSMV), family *Potyviridae* (Stenger et al., 1998), is a *Tritimovirus* that causes yield loss in grass species used as crops, particularly in wheat (*Triticum aestivum*). It was first described in 1919 in the Great Plains as "yellow mosaic" (McKinney, 1937). This plant disease is problematic in wheat growing areas of the world, particularly the Great Plains region of the US, with recent outbreaks in Australia (Ellis et al., 2003) and South America (Truol et al., 2004). WSMV has also been detected in Algeria (Benmokhtar and Yahia, 2009) and Zambia (Kapooria and Ndunguru, 2004).

In Kansas, annual yield loss from WSMV averaged 1-2% for the last decade, equating to an estimated $10-20 million USD loss per annum (Appel et al., 2014). A $16 million USD annual loss was estimated for the state of New South Wales, Australia, after 2006 crop losses from WSMV reached 20,000 hectares in the high rainfall growing areas (Jones and Burges, 2006). Throughout the Great Plains of the United States, WSMV has been identified as the most common cereal grain virus (Burrows et al., 2015). WSMV is vectored by the arthropod wheat curl mite (WCM, *Aceria tosichella* Keifer, (Slykhuis, 1955). Transmission via seed has been recorded at very low rates (Dwyer et al., 2007) and mechanical transmission is used for research purposes (Sánchez-Sánchez et al., 2001, Slykhuis, 1955, Miller et al., 2014).

Biology of WSMV

Understanding the biology of a disease can provide valuable insights into virus-host interactions and virus spread. Plant cells are infected with WSMV virions during feeding of viruliferous WCM (Orlob, 1966). The virus then replicates and moves from infected cells to adjacent ones, spreading through the plant via phloem (Brakke, 1987). WSMV causes leaf symptoms on plants including a yellow-green mosaic and/or leaf streaking (Slykhuis, 1955). This mosaic pattern is due to the interruption of chloroplast development of leaf cells as the plant is systemically infected by the virus (Brakke, 1987). WSMV also causes reduced shoot and root biomass, decreased grain yield, decreased water-use efficiency, and can cause plant death (Pradhan et al., 2015, Price et al., 2010b, Workneh et al., 2010).

WSMV virions are monopartite filamentous rods composed of single-stranded positive sense RNA and a capsid protein with an approximate length of 700 x 13 nm, with concentrations per gram of leaf measuring from 10 to 100 µg (Brakke, 1971). Host cells infected with WSMV may display 'pinwheels' - virus inclusion bodies consisting of aggregations of capsid protein (White and Brakke, 1983) typical of the family *Potyviridae*. The helper component proteinase (HC-Pro) is important in the cycle of WSMV and is involved in mite transmission, viral replication and cell-to-cell and systemic movement through plants (Plisson et al., 2003).

There are four clades of WSMV, determined by coat protein gene sequence (Stenger and French, 2009, Stenger et al., 2002). United States genotypes of WSMV are represented by two isolates, Sidney 81 (WSMV-S81) and Type (WSMV-T) (Stenger et

al., 2002), that share 98.7% polyprotein sequence identity (Choi et al., 2001). WSMV is a relatively new disease in Australia and analysis of 17 coat protein sequences from WSMV sources revealed a likely origin from the Pacific North West of the U.S.A, as the sequences from both locations create a monophyletic cluster within the D1 subclade (Dwyer et al., 2007). Capsid protein sequences of European isolates from France, Italy, Slovakia, and Turkey found a close relationship between the isolates, revealing a monophyletic European group (Gadiou et al., 2009).

A number of detection methods for WSMV are available. A single nucleotide polymorphism (SNP) assay was developed to distinguish WSMV isolates (Rogers et al., 2012). WSMV is routinely detected using enzyme-linked immunosorbent assay (ELISA). Multiplex real-time PCR has also been developed, and is especially suitable when WSMV is found in combination with *Triticum mosaic virus* (TriMV) genus *Poacevirus* and other viruses (Price et al., 2010a) that cannot be visually distinguished. Such viruses include *Wheat mosaic virus* (WMoV), formerly High plains virus (HPV) genus *Emaravirus* (Seifers et al., 1997, Mielke-Ehret and Mühlbach, 2012); *Triticum mosaic virus* genus *Poacevirus* (Seifers et al., 2009, Tatineni et al., 2009); and *Brome streak mosaic virus* genus *Tritimovirus* (BrSMV) (Götz and Maiss, 1995). All of these viruses are vectored by the WCM.

The mechanisms of virus transmission in WCM are not well understood. The traditional categories for viral transmission are non-persistent, semi-persistent, circulative propagative, and circulative non-propagative (Ng and Falk, 2006). Transmission of WSMV via WCM was originally characterized as semi-persistent (Paliwal, 1980), as the

acquisition access period prior to transmission is at least 15 min and there is no latency period (Orlob, 1966). However, WSMV could also be considered a circulative virus as it is transmitted transstadially, remaining with WCM from molt to adult (Siriwetwiwat, 2006) and can remain transmissible for 7 to 61 days, dependent on temperature. Cooler temperatures allow mites to remain infective longer (Orlob, 1966). WSMV is not transmitted transovarially, through the egg stage (Siriwetwiwat, 2006), and the transmission efficiency of WCM decreases with mite age (Orlob, 1966).

Biology of the Wheat Curl Mite

From the *Eriophyoidea* superfamily, WCM are small, approximately 200 μm in length and 75 μm wide (Keifer, 1938) and have a white cigar-shaped body with thanosomal rings and four anterior legs (Slykhuis, 1955, Lindquist, 1996). Mites survive approximately 8-10 days (Somsen and Sill, 1970), and lay approximately 12 to 20 eggs during their lifetime (Del Rosario et al., 1958). The mite life cycle consists of an egg stage, 2 nymph stages, and an adult stage with molts after each nymphal phase (Staples and Allington, 1956). Mite reproduction occurs both by parthenogenesis (Helle and Wysoki, 1983) and by female-produced eggs being fertilized by spermatophores left by males on plant leaves (Oldfield, 1970).

Mite feeding can cause direct damage to wheat but virus transmission to the crop is more consequential. WCM pierce the plant cuticle with their stylet and feed on bulliform cells (Sabelis and Bruin, 1996). This feeding causes yield loss, with an approximate loss of 1-15% in wheat (Harvey et al., 2000, Harvey et al., 2002). Mite

feeding also results in curling of leaves, hence the name of the organism (Slykhuis, 1955, Somsen and Sill, 1970). Mites can move on the leaf approximately 5 cm per hour (Del Rosario et al., 1958) and can be transported on aphids (Gibson and Painter, 1957) and other insects including thrips (*Mary Burrows, MSU, personal communication, 2015*). WCM require a green, living host plant for their survival. When the host plant starts to die, mites move to the tip of the leaf and use their caudal sucker to hold themselves upright and detach (Nault and Styer, 1969), moving passively via wind currents (Slykhuis, 1955, Staples and Allington, 1956, Nault and Styer, 1969) During dispersal, WCM are able to survive for a few days without a host, surviving up to 106 hours when temperatures are cooler (~10°C) and relative humidity is nearly saturated (~95%) (Wosula et al., 2015).

Taxonomy of WCM

The classification of WCM has changed significantly over time. Taxonomists originally classified WCM with a separate mite species that infested onions, garlic and tulips - the dry bulb mite *Aceria tulipae*, due to similar morphology, until the late 1960s. Most eriophyid mites are considered host specific (Oldfield and Proeseler, 1996) - with wheat curl mites as an exception - and to have one species of mite living on members of both Liliaceae and Poaceae was perplexing (Navia et al., 2013). On closer inspection, it was found that the eriophyid mite that inhabits wheat was a separate species from the dry bulb mite, initially distinguished from *Aceria tulipae* as *Aceria tritici* Shevtchenko (Shevtchenko et al., 1970) and later, *Aceria tosichella* Keifer (Amrine and Stasny, 1994).

The mite species differ in size of the genital cover flap, number of annuli of the hysterosoma, organ size, and in developmental growth dimensions (Shevtchenko et al., 1970). Many researchers still referred to WCM as *Aceria tulipae* well into the 1990s. Papers referring to *Aceria tulipae* cited herein are papers using the erroneous name for WCM. Recent molecular work has suggested the possibility of WCM being a complex of species, revealing distinct evolutionary lineages not revealed with morphological studies (Skoracka et al., 2012). Multiple WCM biotypes were described after differences in virulence to sources of wheat resistance were discovered amongst US populations (Harvey et al., 1999). Ability to transmit viruses also varies amongst WCM groups (Seifers et al., 2002), and morphological differences have been noted (Sukhareva, 1981).

Grass Host Range of WCM

In general, eriophyid mites are host specific to one species or a few plant species in the same genus (Oldfield and Proeseler, 1996), but WCM are the exception to this rule as close to 90 plants have been reported as hosts (Sabelis and Bruin, 1996, Amrine and Stasny, 1994, Navia et al., 2013). Most of these are annual grass species. Crops that are hosts of WCM include wheat, barley (*Hordeum vulgare*), corn (*Zea mays*), oats (*Avena sativa*), pearl millet (*Pennisetum glaucum*), rye (*Secale cereale*), and sorghum (*Sorghum bicolor*) (Navia et al., 2013, Seifers et al., 1996). Plants that are hosts for WCM are not always ideal hosts for WSMV, and vice versa. In terms of crop species, wheat is the most impacted by WSMV although yield loss varies among varieties (Miller et al., 2014). Some isolates of WSMV infect barley (*Hordeum vulgare*), oats (*Avena sativa*), and

certain varieties of corn (*Zea mays*) and millet (*Pennisetum glaucum*) are susceptible to WSMV (Brakke, 1971).

The diversity of potential hosts from WCM and WSMV may facilitate disease transmission among crop fields and cropping seasons. Alternative hosts are plants that can support vector growth and reproduction, virus replication, and vector competence, but are not the primary crop host of the disease. These include native grass species, weeds, and volunteer and planted crops (Christian and Willis, 1993, Ito et al., 2012). Cheatgrass (*Bromus tectorum*), also called downy brome and drooping brome, was found in Montana to be the most prevalent grassy weed host for the disease (Ito et al., 2012). Continents, countries, states, and counties have unique flora and cropping systems. Control methods must be tailored to climate, cropping system, and alternative host species in areas of infestation for holistic disease management. If growers know their highest risk alternative host species, they can target management to those species.

Control of WCM and WSMV

Cultural control methods are the most effective for management of WCM and WSMV. In the Great Plains, these include late planting of fall crops such as winter wheat and early planting of spring crops such as spring wheat to avoid mite infestation due to cooler temperatures (Hunger et al., 1992). The most effective method of cultural management is termination of the green bridge, living plant material such as grassy weeds and volunteer wheat in a system that can harbor mites and virus. When seeds are knocked out of the heads of a maturing crop and results in germination of new plants, a

'volunteer' crop, green material is present for the WCM. This is exacerbated by hail and pest issues including wheat stem sawfly (*Cephus cinctus* Norton) (De Corby et al., 2009), birds (Janzen, 1971), and other pests. Hail events close to harvest time intensify the volunteer wheat problem (Anderson and Soper, 2009), and can lead to severe WSMV outbreaks (Staples and Allington, 1956, Thomas and Hein, 2003). Volunteer wheat control can be achieved by tillage, glyphosate and paraquat application (Thomas et al., 2004, Jiang et al., 2005). If the window of time between harvesting wheat and planting winter wheat is less than two weeks, tilling volunteer wheat is better at preventing WCM spread than spraying plants with herbicide (Thomas et al., 2004).

Host resistance can be used in WSMV control, but does not provide complete protection. Natural sources of resistance to WSMV (genes *Wsm1, Wsm2,* and *Wsm3*) are available from the tertiary gene pool from the species intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & DR Dewey). *Wsm1* has been incorporated into commercially available cultivar Mace, a hard red winter wheat (Graybosch et al., 2009); *Wsm2* has been used in RonL, a hard white winter wheat (Seifers et al., 2007); and *Wsm3* is incorporated into wheat germplasm but not yet into commercial cultivars (Zhang et al., 2015). Resistance breakdown occurs when temperatures exceed 24°C for RonL (Seifers et al., 2006) and 27°C for Mace (Seifers et al., 1995). Additionally, a yield penalty is associated with *Wsm1* in the absence of WSMV (Baley et al., 2001, Sharp et al., 2002). Variety TAM 107 incorporated WCM resistance, and was used in Kansas since the 1980s. However, resistance was overcome in the mid-1990s (Harvey et al., 1995, Harvey et al., 1997a) and the variety is no longer effective for disease management.

Preventative control measures, such as use of resistant varieties to WCM and WSMV, late planting, and green bridge removal are particularly useful if vector and/or disease pressure is a high risk. Prevention is effective, however there are currently no options to control WSMV and WCM once established in a field. Identifying new, readily available chemical treatments for vector control could provide options for vector management after planting, to supplement cultural control methods.

Insecticides and Acaricides for WCM Control

Literature regarding WCM chemical control exists for the treatments available 30 to 40 years ago (Harvey et al., 1979), however all effective pesticides have since been deregistered for use on wheat in the US. Previously registered chemicals that showed efficacy at reducing populations of WCM were carbamates (carbofuran, aldicarb) and organophosphates (terbufos); both inhibited acetylcholine esterase, an enzyme involved in neurotransmission. The systemic insecticide carbofuran controls WCM populations when applied to winter wheat seed, and also decreased the incidence of WSMV in the following spring compared to untreated controls (Harvey et al., 1979). However, this pesticide is no longer registered for use on consumable food crops in the United States (EPA, 2011). The organophosphate disulfotan did not reduce WCM due to spring or fall application on seed (Harvey et al., 1979). Insecticides to control WCM in corn were also ineffective to be used in wheat. The exception was the organophosphate terbufos (trade name Counter 15CR) a granular insecticide applied at planting (Fritts et al., 1999). This use was not economical and the product is not labeled for use on wheat. Imidacloprid, a systemic neonicotinoid insecticide, had no effect on WCM populations or WSMV

incidence in the greenhouse or field (Harvey et al., 1997b), but is used to control aphids and thrips during WCM studies.

Economic Considerations of Chemical Control

WSMV impacts gross revenue of wheat and barley crops by reducing grain yield, forage production, and water use efficiency (Velandia et al., 2010). The extent to which revenue is impacted is dependent on destruction the disease causes, which is in turn dictated by timing of infection and susceptibility of the crop as well as environmental conditions. To comprehend the additional cost of insecticides and acaricides for WSMV control via vector control, the additional costs need to be incorporated in profit calculations. Efficacy of treatments is critical, so that chemical costs are worthwhile. This study uses an insecticide as an example of cost to spray, and how these costs will impact producers' profit margins, in a dryland spring wheat crop grown for grain in Montana.

Predictors of Disease Reservoir Potential

As WSMV is identified in new countries, an understanding of how the disease spreads and persists in the environment on disease reservoirs can be critical in understanding how to prevent its persistence. Disease reservoirs are sources (i.e. alternative host plants) of disease inoculum (mite and virus) with potential to harbor inoculum for disease outbreaks. The WCM and WSMV pest complex involves disease reservoirs because the disease needs living plant material for its survival (Somsen and Sill, 1970). Three key parameters determine if a plant will be a good disease reservoir for a viral disease. The host must support vector population reproduction and growth, be

susceptible to infection with the virus, and have the ability to support the proliferation of transmissible virus (Cronin et al., 2010). If one parameter estimate is zero, a plant species cannot act as a reservoir. If no reservoirs are available in a field or neighboring fields, the risk of re-infection in successive years will be greatly reduced. Identifying the phenotypic traits that make plants ideal reservoirs provides a framework for control.

Host lifespan is an important factor in determining propensity of a species to be a disease reservoir. In surveys conducted of weeds and native grasses, WSMV and WCM were found on a higher proportion of annual species (Table 3.01). Host lifespan impacts the makeup of plant leaves, with physiology falling on a continuum of quick return to slow-return phenotypes. Insect vectors, in general, have higher reproductive rates and feed more often on short-lived, quick return hosts (Hogenhout et al., 2008) with rapidly growing leaves containing higher phosphorous concentrations, nitrogen concentrations and metabolic rates than long-lived, slow return phenotypes (Diaz et al., 2004, Wright et al., 2004). One theory for these plant strategies is a growth-defense trade-off, with different genera utilizing different tactics (Fine et al., 2006).

Geographic origin is also a factor contributing to the potential of a species to host WSMV and WCM, as a proxy for time during which the host and pathogen have co-evolved. Introduced species are often poorly defended against the threats in a system due to less exposure and resistance compared to native species of a system (Blumenthal, 2006). For *Barley yellow dwarf virus* (BYDV) and its aphid vector, the only reservoir trait not explained by host lifespan, geographic origin and phylogeny was host competence (Cronin et al., 2010). Comparing plants of different phylogenetic

background, US provenance, and life cycle length for the three reservoir qualities will provide insight into the evolution and ecology of WCM and WSMV host ranges and disease outbreaks.

Summary and Research Goals

As the WCM and the viruses it vectors, including WSMV, becomes an increasing global problem, and control methods are limited to cultural methods, new tools to fight this disease are required. This study aimed to clear up misconceptions - ascertained through survey data- amid Montana and Texas farmers who reported spraying insecticides on wheat to control WCM, when none were registered for WCM management on wheat (*Mary Burrows, MSU, personal communication, 2014*). The intent of this study was to halt ineffective insecticide sprays by providing efficacy data on acaricide options available in the marketplace. The research goals of this project were 1) To test common insecticides and acaricides belonging to diverse modes of action for their efficacy against WCM in Montana and 2) to assess WCM and WSMV susceptibility of grass species by provenance and life history to predict their disease reservoir potential.

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EVALUATING PESTICIDES FOR EFFICACY AGAINST THE WHEAT CURL MITE ON SPRING AND WINTER WHEAT IN MONTANA

Abstract

Wheat streak mosaic virus (WSMV) is the most common cereal grain virus in the Great Plains and current controls for the disease rely on cultural methods. There are no chemical treatments available for the WSMV vector, the wheat curl mite (WCM, *Aceria tosichella* Keifer). These experiments tested carbamate, organophosphate, pyrethroid, neonicotinoid, biological control, oil, ovicide, mite growth inhibitor, and soap treatments for potential to decrease WCM population growth on spring wheat (cv. Choteau). Treatment with the active ingredients aldicarb and chlorpyrifos decreased greenhouse WCM population growth rates compared to untreated controls ($p<0.001$ and $p<0.001$, respectively). Aldicarb is no longer registered for use, whereas chlorpyrifos shows potential for WCM chemical control. Field trials included spring wheat in 2013 and winter wheat in 2013-2014 testing organophosphate, pyrethroid, oil, soap, and mite growth inhibitor treatments. WCM incidence was not affected by treatment relative to controls in 2013 spring wheat ($p=0.32$) and disease presence was too low in the 2013-2014 winter wheat trial to see a treatment effect ($p=0.78$). Chlorpyrifos reduces WCM populations and with further study may provide an additional tool for integrated control of WSMV.

Introduction

Wheat streak mosaic virus (WSMV), a *Tritimovirus* in the family *Potyviridae* (Stenger et al., 1998), causes economic loss in wheat growing regions of the world. WSMV is vectored by the wheat curl mite (WCM, *Aceria tosichella* Keifer) (Slykhuis, 1955), a microscopic eriophyid mite. There are no chemical treatments available for WCM control. Misconceptions about treatments are common, due to limited research on the matter and confusion with other mite genera. Sulfur has no efficacy against WCM (Conner et al., 1991), and many people still treat greenhouses with sulfur to prevent or manage WCM (Li et al., 2002, Conner et al., 1991, Li et al., 2007, Lanoiselet et al., 2008). Sulfur, lime-sulfur sprays and sulfur dusts have been shown to be effective on other eriophyid mites, such as bud mites (*Eriophyes*), but not *Aceria* (Jeppson et al., 1975). Aldicarb, a carbamate, has known efficacy against the WCM and other eriophyid mites (Conner et al., 1991), but is no longer registered for use in the US due to toxicity to mammals. Aldicarb has systemic action in plants that inhibits an enzyme associated with neurotransmission, cholinesterase, leading to paralysis and death (Kolbezen et al., 1954), the same mode of action as organophosphates.

Organophosphates and carbamates have had variable efficacy in controlling WCM, and those that work are no longer available for use in the U.S.A. Acephate, chlorpyrifos, diazinon and malathion have the same mode of action, and both chemical subgroups may have efficacy at reducing WCM populations, as they function the same as aldicarb. In field trials, the carbamate carbofuran controlled WCM populations when applied to winter wheat seed, and also decreased the incidence of WSMV the following

spring compared to untreated controls (Harvey et al., 1979). However, this product has not been an option for farmers in the United States since 2009, when the Environmental Protection Agency (EPA) delisted carbofuran-containing products (trade names "Furadan" and "Curater") for crops grown for human consumption due to toxicity concerns (EPA, 2011). When tested under field conditions, the active ingredient disulfoton (an organophosphate) was ineffective at controlling WCM (Harvey et al., 1979). Corn (*Zea mays*) is one host for the WCM (Sill and Connin, 1953), and insecticides for WCM control on corn such as carbofuran (Furadan 4F), phorate (Thimet 20G) and disulfoton (Disyston 15G) were ineffective at controlling WCM (Fritts et al., 1999). However, the organophosphate terbufos (Counter 15CR) showed some efficacy when applied at planting (Fritts et al., 1999). Terbufos applications are not economically viable (Fritts et al., 1999) and this product is only labeled for corn, sugar beets and sorghum in the U.S.A.

Other mode of actions have not proven effective for WCM control, such as neonicotinoids. The neonicotinoid product imidacloprid, a systemic insecticide targeting the nicotinic acetylcholine receptor agonists, had no effect on WCM populations or WSMV incidence in the greenhouse or field (Harvey et al., 1997b). The chemical is widely used in field crops, and is useful for controlling aphids and thrips (Elbert et al., 1998). Onion thrips (*Thrips tabaci*) and western flower thrips (*Frankliniella occidentalis*) have been shown to feed on WCM (Stilwell, 2009), and imidacloprid treatment increased WCM populations when applied for insect control (Harvey et al., 1997b) due to removing natural mite predators (Kunkel et al., 1999). Removing predators

of the WCM is one example of detrimental unintended side effects of unnecessary chemical sprays due to misinformation and another reason why research into WCM chemical control is so important.

Organic farming cannot utilize synthetic chemicals such as carbamates and organophosphates. Organic products for WCM control have not been reported in the literature. Products for organic farming systems also need to be tested for efficacy against the WCM including products that halt mite feeding, prevent reproduction and induce plant defense responses. Oils such as neem oil are an alternative to synthetic pesticides that stop insects and mites from feeding, laying eggs or reproducing (Attri and Prasad, 1980). Neem oil reduced populations of the coconut eriophyid mite (*Aceria guerreronis* Keifer) by 34% (Pushpa and Nandihalli, 2008). The WCM is in the same genus, and it is reasonable to postulate that WCM population growth may be reduced with neem oil.

Although alternatives to synthetic pesticides could provide control of the WCM, cost to spray calls into question the usefulness of these alternative controls on a low-input, low-value crop. Issues arise due to adverse environmental effects, vector resistance to chemicals (Castle et al., 2009, Mouchet, 1988), and removal of beneficial organisms from a system (Nash et al., 2008).

In summary, previous work with chemical controls for the WCM is limited and there are currently no pesticides available on the market for its management. This work was completed to discover if any mode of action or alternatives to synthetics had efficacy on the WCM and could be incorporated into a control strategy for WCM when WSMV is present. It is also important for providing results that can refute misconceptions

concerning efficacy of products like sulfur. This study evaluated 1) efficacy in the greenhouse of chemical treatments with varying mode of action against WCM, including Organic Materials Review Institute (OMRI)-approved products and 2) efficacy in the field of 10 treatments on spring and winter wheat against WCM population and WSMV incidence.

Materials and Methods

Mite Population and Virus Isolate

Mites used in greenhouse experiments were sourced from a colony established from WCM collected in September 2007 from the Arthur H. Post Agronomy Research Farm, Bozeman MT. The colony was maintained in a plant growth chamber at 18-24°C as described by Ito *et al*, 2012. The population was fortified with WCM collected from Gallatin County, MT, to keep it representative of the local WCM population. Mite populations were originally grown on a mixed population of susceptible spring wheat cultivars: Amidon, Fortuna, and Choteau and later only on Choteau, in MSU mix soil (described above). Every few months, wheat plants of the WCM population were re-inoculated with WSMV isolate Conrad-I (stored at -80°C), originally collected in 2007 from symptomatic winter wheat in Conrad, MT (Ito et al., 2012). To mechanically transmit the virus to the wheat plants, a 1:10 mixture of macerated infected leaf tissue and phosphate buffered saline (PBS; pH 7.2, 136.9mM NaCl, 8.1mM Na_2HPO_4, 1.5 mM of KH_2PO_4 and 2.9mM KCl), plus 1% of carborundum (320 grit) to abrade the leaf surface, were combined in a plastic extraction bag (Agdia, Inc., Elkhart, IN). Using gloved

fingertips, the virus mixture was gently rubbed onto wheat leaves. Source plant leaves were tested for WSMV using ELISA 2-3 weeks after inoculation to check WCM populations were viruliferous.

Mite Population Growth Rate

Population growth rate was used to determine whether WCM numbers on a whole plant were increasing, decreasing or remaining static under treatments in greenhouse experiments. Mites were determined to be living based on movement and white color, and eggs were not included in the count. The Malthusian growth model was used as the basis of calculations (Yaninek et al., 1989); $P(t) = P_0 e^{rt}$ where P_0 is the number of mites applied initially, $P(t)$ is the number of mites after treatment, 'r' is the population growth rate and 't' is time (in days) that mites were on plants before the final mite count and subsequent experiment termination. Population decline was indicated by $r<0$ and an increasing population $r>0$. When no mites were found, a value of 0.001 was used in place of zero for the P(t) value (the final number of mites), to allow for log transformation in the mite population growth rate formula.

Greenhouse Experiments

Five experiments were conducted in the greenhouse to test insecticides, biological controls, organic treatments, and seed treatments for efficacy against the WCM. For these experiments, wheat seeds (*Triticum aestivum* L. cv. Choteau) were planted two per pot (12.7cm diameter pots 9cm height) in MSU mix (1:1:1 Canadian sphagnum peat moss, mineral soil mix and Aquagro 2000G [Aquatrols, NJ]) and kept under greenhouse

conditions at 24 ± 4°C (day) and 18 ± 4°C (night), photoperiod of 16 h and 8 h (day and night, respectively). The variety Choteau was chosen for its susceptibility to both WCM and WSMV. Pots were thinned to one plant per pot at growth stage DC 12 (Two leaves emerged) (Zadoks et al., 1974), with individual wheat plants considered an experimental unit. WCM were transferred to experimental plants from the WCM colony (described above) using the leaf piece method at growth stage DC 13-14 (three to four leaves emerged) (Zadoks et al., 1974), by attaching leaves 6-7 cm in length holding 30-50 WCM to the plants with paper clips on the adaxial surface of leaves. Leaf pieces were attached close to the base of stems on the youngest expanded leaf. The number of WCM on each leaf piece was recorded. Plants were enclosed in a plastic cover (14 cm height, 8.5 cm diameter, Pro-Kal containers, Michigan) with three window cut-outs (5cm x 2cm) sealed with a fine nylon lab pak mesh (25µm; Sefer AG, Switzerland). After 2 d, leaf pieces were removed and the number of WCM remaining on the leaf piece were counted to get an estimate of WCM number transferred to the experimental plant. Plants were left in the greenhouse for two weeks before chemical treatments were applied. For the seed treatment trial, chemicals were applied prior to planting.

Insecticide Experiment

Efficacy of organophosphate (Acetylcholinesterase inhibitors), carbamate (Acetylcholinesterase inhibitors), and pyrethroid (sodium channel modulator) products (Table 2.01) for reducing WCM populations was tested on spring wheat plants, grown and infested with WCM as described above. The experiment was a completely randomized design with eight replications per treatment and conducted three times.

Included in each experiment was an untreated, WCM infested control group, and seven chemical treatments.

Table 2.01. Insecticide treatments tested in the greenhouse

#	Active ingredient	Mode of action	Trade name	Active ingredient amount	Supplier
1	Control	Control	Control	NA	NA
2	Methomyl	Carbamate (1A)	Lannate LV	163 g a.i./ha	Dupont
3	Aldicarb	Carbamate (1A)	Temik	185 g a.i./ha	Bayer
4	Dimethoate	Organophosphate (1B)	Dimethoate 400	439 g a.i./ha	Loveland products
5	Chlorpyrifos	Organophosphate (1B)	Lorsban 4E	526 g a.i./ha	Dow Chemical
6	Chlorpyrifos	Organophosphate (1B)	Lorsban advanced	471 g a.i./ha	Dow Chemical
7	Zeta-cypermethrin* S-Cyano methyl	Pyrethroid (3A)	Mustang Max	27 g a.i./ha	FMC Ag. Solutions
8	Lambda-cyhalothrin	Pyrethroid (3A)	Warrior II	34 g a.i./ha	Syngenta

All treatments, except aldicarb, were applied using a chemical spray booth (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN) calibrated to run at 3.78 kph, spraying a length of 1.8 m, 275 kPa pressure with nozzle height 35 cm above the top of the plant. A TeeJet 8002VS nozzle was used for application and pesticides were prepared and mixed with water to make up 500 mL of solution. The eight plants treated with aldicarb had the granular product applied to the soil and watered in, according to label directions. Active ingredient amount selected for each treatment were the highest labeled rates acceptable for wheat crops (Table 2.01). The only treatment not labeled for wheat and no longer registered for use was aldicarb. Three to four days after

treatment, mite numbers on the whole plant were calculated by counting WCM under the dissecting microscope at 10x magnification.

Chlorpyrifos Experiment

Two subsequent efficacy trials of chlorpyrifos treatments, Lorsban 4E and Lorsban advanced (described in Table 2.01) were conducted to confirm results discovered in the insecticide experiment. The ability of Lorsban products to reduce WCM populations was tested on spring wheat plants, grown and infested with WCM as described above. The experiment was a completely randomized design with eight replications per treatment and conducted two times. Included in each experiment was an untreated, WCM infested control group, and two chemical treatments. Chlorpyrifos treatments were applied using a chemical spray booth (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN) calibrated to run at 3.78 kph, spraying a length of 1.8 m, 275 kPa pressure with nozzle height 35 cm above the top of the plant. A TeeJet 8002VS nozzle was used for application and chemicals were prepared and mixed with water to make up 500 mL of solution. Three days after treatment, mite numbers on the whole plant were calculated by counting WCM under the dissecting microscope at 10x magnification.

Biological Control Experiment

Efficacy of biocontrol, mite growth inhibitor, and ovicide products (Table 2.02) were analyzed for potential to reduce WCM populations on spring wheat plants. The experiment was a completely randomized design with eight replications per treatment and

conducted three times. Included in each experiment was an untreated, WCM infested control group and five other treatments. Treatments were applied using a chemical spray booth (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN) calibrated to run at 3.78 kph, spraying a length of 1.8 m, 275 kPa pressure with nozzle height 35 cm above the top of the plant. A TeeJet 8002VS nozzle was used for application and pesticides were prepared and mixed with water to make up 500 mL of solution. Five DAT, mite numbers on the whole plant were calculated by counting WCM under the dissecting microscope at 10x magnification. Extract of neem oil (trade name "Trilogy", Table 2.02) and *Bacillus mycoides* isolate J are available for use on wheat crops. Hexythiazox, etoxazole, and petroleum distillate are not labeled for wheat.

Table 2.02. Biological controls, mite growth inhibitors and miticides tested in the greenhouse

#	Active ingredient	Description	Trade name	Active ingredient amount	Supplier
1	Control	Control	Control	NA	NA
2	Hexythiazox	Mite growth inhibitor (10A)	Onager	156 g a.i./ha	Gowan
3	Etoxazole	Mite growth inhibitor (10B)	Zeal	157 g a.i./ha	Valent
4	*Bacillus mycoides* J	Bacterial biocontrol	BmJ	$1.4*10^{10}$ spores/ha	Montana Microbial Products
5	Petroleum distillate	Ovicide	Sunspray	4883 g a.i./ha	Sunoco
6	Extract of neem oil	Miticide	Trilogy	3460 g a.i./ha	Certis USA

Organic Experiment

A range of organic agricultural products (Table 2.03) were tested for potential to reduce WCM population growth rates on spring wheat plants, grown and infested with

WCM as described above. The experiment was a completely randomized design with eight replications per treatment and conducted three times. Included in each experiment was an untreated, WCM infested control group and six organic treatments. The sulfur treatment was prepared by pulverizing the granules, dissolving in water and filtering with cheesecloth before making up the 500mL bottle for application.

Table 2.03. Organic products tested in the greenhouse

#	Active ingredient	Description	Trade name	Active ingredient amount	Supplier
1	Control	Control	Control	NA	NA
2	Chitosan	Stimulates plant defense	Chitosan	825 g a.i./ha	Sigma Aldrich
3	Neem oil	Oil antifeedant	Debug turbo	130 g a.i./ha	Agro logistic
	Azadirachtim	Miticide		1.4 g a.i./ha	
4	Potassium salts	Organic soap insecticide	Des-X	2323 g a.i./ha	Certis USA
5	*I.fumosorosea* Apopka	Organic microbial insecticide	PFR-97	449 g a.i./ha	Certis USA
6	Potassium silicate	Organic insecticide / miticide	Sil-matrix	717 g a.i./ha	Certis USA
7	Sulfur	Contact poison	Sulfur	1156 g a.i./ha	Sigma Aldrich

Treatments were applied using a chemical spray booth (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN) calibrated to run at 3.78 kph, spraying a length of 1.8 m, 275 kPa pressure with nozzle height 35 cm above the top of the plant. A TeeJet 8002VS nozzle was used for application and pesticides were prepared and mixed with water to make up 500 mL of solution. Active ingredient amount selected for each treatment were the highest labeled rates acceptable for wheat crops (Table 2.01). Treatments not labeled for wheat are chitosan and *I.fumosorosea*. Three to four DAT,

mite numbers on the whole plant were calculated by counting WCM under the dissecting microscope at 10x magnification.

Seed Treatment Experiment

A thiamethoxam neonicotinoid seed treatment (Cruiser Maxx for cereals®, Syngenta, Pasco, WA) was tested at two different rates (Table 2.04) for ability to reduce WCM populations on spring wheat plants. Treatment was applied prior to planting by the Syngenta chemical representative by making a slurry diluted in water and applying with seed treatment equipment. Chemical rates were determined by Syngenta's recommendations.

Table 2.04. Seed treatment tested in the greenhouse

#	Active ingredient	Mode of action / Description	Treatment	Active ingredient amount	Supplier
1	Control	Control	Control	NA	NA
2	Thiamethoxam	Neonicotinoid (4A)	Cruiser Maxx	272 g a.i./ha	Syngenta
	Difenoconazole	Fungicide		327 g a.i./ha	
	Mefenoxam	Fungicide		54 g a.i./ha	
3	Thiamethoxam	Neonicotinoid (4A)	Cruiser Maxx	205 g a.i./ha	Syngenta
	Difenoconazole	Fungicide		246 g a.i./ha	
	Mefenoxam	Fungicide		41 g a.i./ha	

Plants were grown and infested with WCM as described above, and mites were left on experimental plants for 14 days. Mite numbers on the whole plant were calculated by counting WCM under the dissecting microscope at 10x magnification. The experiment was a completely randomized design with eight replications per treatment and conducted three times. Included in each experiment was an untreated, WCM infested control group and two rates of the thiamethoxam treatment.

Statistical Analysis of Greenhouse Data

Population growth rate of WCM under chemical treatment were analyzed using R software, version 3.0.2. The assumption of normality was checked using the Shapiro-wilk test and the assumption of homogeneity of variances with Levene's test. A linear mixed effect model was conducted, with treatment as a fixed effect and trial as a random effect, to check for differences between means of treatment groups and interactions between trial and treatment. When p-values were less than 0.05, a Tukey's HSD (honest significance difference) test was conducted to determine treatment differences and compute mean comparison letters.

Spring Wheat Field Trial

Spring wheat (*Tritcum aestivum* cv. Choteau) was planted on June 5, 2013 at the Lutz farm (Blackdog silt loam, pH 7.3), Bozeman, Montana, (coordinates 45.805, -111.045 elevation 4624'). Seeding rate was 60 seeds/ m^2 and planted into 70, 8-row plots (2.75 m x 2.45 m) with buffer regions of 2 m between plots. Field design was a randomized complete block with seven blocks and ten treatments, including an inoculated but untreated control. Nine pesticides (Table 2.05) were chosen based on efficacy in preliminary greenhouse experiments. Blocks were placed to account for a -0.3% east to west slope in the field. The field was fertilized on 24 June, 2013 with 112 kg/ha urea. Bordering strips of wheat beside the experiment were sampled to check for naturally occurring WCM and WSMV on 1 July, 2013, and none was found. Plots were inoculated with viruliferous WCM on 8 July, 2013 at growth stage DC 21 (main shoot and one tiller) (Zadoks et al., 1974). Leaves from the wheat curl mite population housed in the growth

chamber were viewed with a dissecting microscope, 10x magnification, and divided into pieces with approximately 50 WCM per leaf piece. Plots were infested by clipping three leaf pieces to one centrally located plant per plot. Leaf pieces were attached to the adaxial surface of the three youngest fully expanded leaves to allow mites to crawl onto wheat plants. The inoculated plant was marked using a flag and also wrapped at the base with colored tape. Leaf pieces were left on for 2 days before removal.

Pesticides were applied on 25 July, 2013 with weather conditions of clear skies, 25.5°C and wind speed of 0-5 km per hour. Chemical treatments were applied using a CO_2-charged backpack sprayer with 4, 8002VS nozzles spaced one foot apart. Chemicals were applied once, using highest recommended dosage according to chemical labels (Table 2.05). When chemicals were not labeled for wheat, chemical dosages were calculated by choosing closely related plant species and following spray guidelines. Plants were at growth stage DC 39 (flag leaf ligule/collar just visible) (Zadoks et al., 1974). Sampling for WCM and WSMV was conducted on 5 August, 2013, 11 d post-treatment. Twenty five youngest, fully expanded leaves were collected per plot in a systematic manner from the inner 1.75 m and inner 6 rows and stored on ice in 10.2 X 15.2cm plastic sample bags (Fisher Scientific, Pittsburgh, PA). Leaves were checked for mite presence under a dissecting microscope at a magnification of 10x. Leaves were then frozen at -20°C until virus detection with ELISA. Samples were collected of 0.9 m transects of mature wheat from the middle row of each plot on 6 September, 2013, samples were dried for two weeks and yield estimates were calculated from grain weight (g).

Table 2.05. Pesticide applications in a 2013 spring wheat field trial to test efficacy against the wheat curl mite and *Wheat streak mosaic virus* (WSMV) in Bozeman, MT

#	Active ingredient	Mode of action / Description	Trade name	Active ingredient amount	Supplier
1	Control	NA	Control	NA	NA
2	Dimethoate	Organophosphate (1B)	Dimethoate 400	438.62 g a.i./ha	Loveland products
3	Chlorpyrifos	Organophosphate (1B)	Lorsban 4E	525.97 g a.i./ha	Dow Chemical
4	Zeta-cypermethrin* S-Cyano methyl	Pyrethroid (3A)	Mustang Max	26.68 g a.i./ha	FMC Ag. Solutions
5	Lambda-cyhalothrin	Pyrethroid (3A)	Warrior II	33.81 g a.i./ha	Syngenta
6	Hexythiazox	Mite growth inhibitor (10A)	Onager	155.73 g a.i./ha	Gowan
7	Neem oil	Oil antifeedant	Debug turbo	130.09 g a.i./ha	Agro logistic
8	Azadirachtim *I.fumosorosea* Apopka	Miticide Organic microbial insecticide	PFR-97	1.38 g a.i./ha 448.73 g a.i./ha	Certis USA
9	Potassium salts	Organic soap	Des-X	2323.07 g a.i./ha	Certis USA
10	Sulfur	Contact poison	Sulfur	1156.43 g a.i./ha	Sigma Aldrich

Winter Wheat Field Trial

Winter wheat (*Tritcum aesticum* cv. Genou) was planted at the Lutz farm (Blackdog silt loam, pH 7.3), Bozeman, Montana, (coordinates 45.805, -111.045 elevation 4624') on 2 October, 2013. Seed was planted at a rate of 90 seeds/m^{-2} on 2 October, 2013 into 70, 7-row plots (2.75 m x 2.45 m) with buffer regions of 2 m between plots. Field design was a randomized complete block with seven blocks and ten treatments, including an inoculated but untreated control. Nine pesticides (Table 2.06) were chosen based on efficacy in preliminary greenhouse experiments. Blocks were placed to account for a -0.3% east to west slope in the field. The field was fertilized at

planting on 2 October, 2013 with 112 kg/ha starter fertilizer N-P-K: 5.5-26-25. Bordering strips of wheat beside the experiment were sampled to check for naturally occurring WCM and WSMV on 1 May, 2014 and none was found. Plots were inoculated with viruliferous WCM 8 May, 2014 at growth stage DC 21 (main shoot and one tiller) (Zadoks et al., 1974), as described above. Leaf segments were left on for two days before removal.

An initial sampling was conducted on 22 May, 2014 post-inoculation but pre-chemical treatment, from the inner 1.63 m and inner 5 rows of plots, 60 whole leaves were collected per plot (including control plots) and stored on ice in 10.2 X 15.2cm plastic sample bags (Fisher Scientific, Pittsburgh, PA). Thirty of the leaves were checked for mite presence under a dissecting microscope, at a magnification of 10x. Thirty leaves were stored at -20°C to preserve samples for later detection of virus presence using ELISA.

Pesticides (Table 2.06) were applied on 22 May, 2014 at 6 pm in the evening with weather conditions of clear skies, 25°C and wind speed of 0-10 km per hour when plants were at growth stage DC 26 (Main shoot and 6 tillers) (Zadoks et al., 1974). Chemical treatments were sprayed using a CO_2-charged backpack sprayer with four TeeJet 8002VS nozzles spaced one foot apart. Chemicals were applied once, using highest recommended dosage according to chemical labels. When chemicals were not labeled for wheat, chemical dosages were calculated by choosing closely related plant species and following spray guidelines. Two subsequent samplings were conducted on 5 June 2014 and 26 June, 2014; 14 d post-treatment and 35 d post-treatment, respectively. Plots were

harvested on 6 August, 2014 with a research combine (Wintersteiger, Salt Lake City, UT) and plot lengths were measured. Harvested samples were threshed and dried, then grain samples were weighed. Yield estimates (kg/ha) were calculated using harvested grain weights per unit area. Grain was analyzed for protein content, moisture and test weight at the Cereal Quality Lab, Montana State University.

Table 2.06. Pesticide applications in a 2014 winter wheat field trial to test efficacy against the wheat curl mite and *Wheat streak mosaic virus* (WSMV) in Bozeman, MT.

#	Active ingredient	Mode of action / Description	Trade name	Active ingredient amount	Supplier
1	Control	NA	Control	NA	Control
2	Methomyl	Carbamate (1A)	Lannate LV	163.41 g a.i./ha	Dupont
3	Dimethoate	Organophosphate (1B)	Dimethoate 400	438.62 g a.i./ha	Loveland products
4	Chlorpyrifos	Organophosphate (1B)	Lorsban 4E	525.97 g a.i./ha	Dow Chemical
5	Zeta-cypermethrin* S-Cyano methyl	Pyrethroid (3A)	Mustang Max	26.68 g a.i./ha	FMC Ag. Solutions
6	Lambda-cyhalothrin	Pyrethroid (3A)	Warrior II	33.81 g a.i./ha	Syngenta
7	Hexythiazox	Mite growth inhibitor (10A)	Onager	155.73 g a.i./ha	Gowan
8	Etoxazole	Mite growth inhibitor (10B)	Zeal	156.56 g a.i./ha	Valent
9	Neem oil	Oil antifeedant	Debug turbo	130.09 g a.i./ha	Agro logistic
	Azadirachtim	Miticide		1.38 g a.i./ha	
10	Potassium salts	Organic soap	Des-X	2323.07 g a.i./ha	Certis USA

Enzyme-Linked Immunosorbent Assay

To determine WSMV presence in field trials, leaf samples were tested using enzyme-linked immunosorbent assay (ELISA). Using a multi-channel pipette (Thermoscientific Finn pipette 30-300µL) 100ul/well of 1x carbonate buffer (0.05M

sodium carbonate, pH 9.6) was loaded into 96 well microplates (Greiner Bio-One, U-shape, Monroe CA) at least 15 min before leaf samples were loaded. Wheat leaf segments approximately 6 cm in length were enclosed in 10.2 X 15.2cm sample bags (Fisher Scientific, Pittsburgh, PA) and phosphate buffered saline (PBS, 136.9 mM sodium chloride [NaCl], 8.1 mM sodium phosphate dibasic [Na_2HPO_4], 1.5 mM potassium phosphate monobasic [KH_2PO_4] and 2.9 mM potassium chloride [KCl], pH 7.4) was added in a 1:10 dilution (wt:vol) of plant sample to PBS and macerated. Sample bags were stored on ice during processing. Microplates were inverted to remove carbonate buffer before plates were loaded with sample material. Ground plant material (200µl per well) was loaded into wells of two replicated microplates. After loading, microplates were stored in a plastic bag and incubated overnight or up to 4 d at 4°C.

After incubation, plates were inverted to remove plant material and washed using a microplate washer (Tecan hydrospeed, Durham, NC, 30054550) for six cycles of washing and five sec of soak with phosphate buffered saline tween (PBST, PBS with 0.5% Tween 20, [Agdia, Elkhart, IN]). Plates were then loaded with 100 ul/well of a primary antibody solution, prepared immediately before use at a working dilution 1:1000 anti-WSMV rabbit antiserum (American type culture collection [ATCC], Manassas, VA) mixed with a solution of 1 part Superblock (ScyTek Laboratories Inc., Logan, UT) to 7 parts ECI buffer (500 parts PBST, 10 parts Polyvinylpyrrolidone (PVP, Sigma-Aldrich, St. Louis, MO) and 1 part bovine serum albumin 2930 (BSA, EMD chemicals inc., Gibbstown NJ).

After incubation for two hours at room temperatures, plates were washed for four wash cycles and five sec of soak using PBST. Secondary antibody solution was prepared at a working dilution of 1:30,000 of anti-rabbit IgG, whole molecule, alkaline phosphatase affinity isolated antibody produced in goat (Sigma-Aldrich, St. Louis, MO) with one part Superblock and seven parts ECI buffer. Secondary antibody solution (100 uL/well) was loaded into plates and incubated for two h at room temperature. Plates were then washed for four cycles with a five sec soak with PBST.

P-nitrophenol (PNP) buffer was prepared immediately before use and vortexed for two minutes in an aluminum foil-covered Falcon tube (BD bioscience, San Diego, CA). The solution was one PNP substrate tablet (Agdia, Elkhart, IN) per 5 mL of buffer solution (0.5mM magnesium chloride hexahydrate ($MgCl_2(H_2O)_6$) and 1.0 M diethanolamine ($C_4H_{11}NO_2$), pH 9.8). PNP running solution (100 uL/well) was added to each well and plates were stored in the dark for 2 h. Plates were analyzed on a spectrometer microplate reader (SpectraMax Plus, Molecular devices, Sunnywyle, CA) at wavelengths of 405 and 450 nm to quantify absorbance of the color reaction.

Each microplate had one positive standard and at least four negative standards, with negative standards distributed systematically across the microplate. Standards were prepared for each plate, using a 1:10 dilution of leaf tissue with PBS buffer as described above. Positive standards were symptomatic, greenhouse-grown spring wheat cv. Choteau mechanically inoculated with the Conrad I WSMV strain and stored frozen at -20°C. Negative controls were uninoculated, virus-free spring wheat plants cv. Choteau reared in the greenhouse. Each plate also had one well with PBS buffer solution, to check

background. Negative standards were averaged and individual samples were considered infected with WSMV if their absorbance value was two standard deviations higher than the absorbance value of negative standards.

Statistical Analysis of Field Data

Proportion of leaves infected with WSMV per plot, total number of WCM per plot, and yield data were analyzed using R software, version 3.0.2. The assumption of normality was checked using the Shapiro-wilk test and the assumption of homogeneity of variances with Levenne's test. Proportion of leaves infected with WSMV per plot was logit transformed to meet assumptions, with untransformed data displayed. ANOVA for a complete randomized block design was conducted, with block as the error term, to check for differences between means of treatment groups. When p-values were less than 0.05, a Tukey pairwise comparison test was conducted. Yield estimates (kg/ha) for the spring wheat field trial in 2013 were calculated from samplings of grain from 0.9 m sections within plots. Yield estimates (kg/ha) for the winter wheat field trial in 2014 were calculated from total plot grain yield.

Economic Analysis

This study considers how insecticide costs will impact wheat producers' profit margins, with dryland hard red spring wheat grown in Montana as an example. The impact of cost to spray insecticides for WCM control was considered using a linear equation for profit, $P(x) = R(x) – C(x)$, where P is grain profits, R is total crop revenue and C is insecticide costs. Chemical costs are dictated by the price of the product,

chemical spray rates (chemical labels vary between states in the US), number of sprays per growing season, labor costs, and equipment costs.

Results

Greenhouse Insecticide Experiment

In the insecticide experiment with carbamate, organophosphate, and pyrethroid treatments, there was a difference between treatments ($p<0.001$). Untreated control WCM populations had positive growth (Table 2.07). Aldicarb (trade name "Temik) and chlorpyrifos (Trade name "Lorsban 4E" and "Lorsban advanced") treatments reduced WCM populations compared to the control.

Table 2.07. The effect of carbamates, organophosphates and pyrethroids on population growth rate of WCM

#	Active ingredient	Mode of action	Trade name	Average population growth rate (n=24)[y]	SE[z]
1	Control	NA	NA	0.043 a	0.014
2	Methomyl	Carbamate (1A)	Lannate LV	-0.029 ab	0.014
3	Aldicarb	Carbamate (1A)	Temik	-0.177 bc	0.027
4	Dimethoate	Organophosphate (1B)	Dimethoate 400	-0.034 ab	0.033
5	Chlorpyrifos	Organophosphate (1B)	Lorsban 4E	-0.298 c	0.061
6	Chlorpyrifos	Organophosphate (1B)	Lorsban advanced	-0.180 bc	0.047
7	Zeta-cypermethrin*	Pyrethroid (3A)	Mustang Max	-0.058 ab	0.026
8	S-Cyano methyl Lambda-cyhalothrin	Pyrethroid (3A)	Warrior II	-0.097 ab	0.047

[y] Means within each column followed by the same letter are not different from each other at P = 0.05.
[z] SE, standard error.

Greenhouse Chlorpyrifos Experiment

In the subsequent insecticide experiment with active ingredient chlorpyrifos products, there was a difference between treatments ($p<0.001$). Untreated control WCM populations had positive growth (Table 2.08). Chlorpyrifos (Trade name "Lorsban 4E" and "Lorsban advanced") treatments reduced WCM populations compared to the control. There was no interaction between trial and treatment ($p=0.355$).

Table 2.08. The effect of active ingredient chlorpyrifos on population growth rate of WCM in a subsequent experiment.

#	Active ingredient	Mode of action	Trade name	Average population growth rate[y]		SE[z]
1	Control	NA	NA	0.051	a	0.010
2	Chlorpyrifos	Organophosphate (1B)	Lorsban 4E	-0.251	b	0.050
3	Chlorpyrifos	Organophosphate (1B)	Lorsban Advanced	-0.308	b	0.056

[y] Means within each column followed by the same letter are not significantly different from each other at P = 0.05.
[z] SE, standard error.

Greenhouse Biological Control Experiment

For the biological controls, mite growth inhibitors, and ovicides there was no difference between treatments ($p=0.09$, Table 2.09). Control plants had a positive population growth rate, as did plants treated with extract of neem oil (trade name "Trilogy").

Table 2.09. The effect of biological control, plant defense response initiator, mite growth inhibitor, and ovicide treatment on population growth rate of WCM

#	Active ingredient	Mode of action / Description	Trade name	Average population growth rate (n=24)[y]	SE[z]
1	Control	NA	NA	0.047 a	0.013
2	Hexythiazox	Mite growth inhibitor (10A)	Onager	-0.045 a	0.045
3	Etoxazole	Mite growth inhibitor (10B)	Zeal	-0.057 a	0.048
4	*Bacillus mycoides* J	Bacterial biocontrol	BmJ	-0.002 a	0.031
5	Petroleum distillate	Ovicide	Sunspray	-0.059 a	0.047
6	Extract of neem oil	Miticide	Trilogy	0.028 a	0.028

[y] Means within each column followed by the same letter are not different from each other at P = 0.05.
[z] SE, standard error.

Greenhouse Organic Experiment

There was a difference in population growth rate of WCM under sulfur and potassium salt treatments (Table 2.10, p=0.002), and marginal differences between the untreated control and potassium salt and neem oil with azadirachtim treatments.

Table 2.10. The effect of organic products on population growth rate of WCM

#	Active ingredient	Description	Trade name	Average population growth rate (n=24)[y]		SE[z]
1	Control	NA	NA	0.020	ab	0.017
2	Chitosan	Stimulates plant defense	Chitosan	0.003	ab	0.016
3	Neem oil Azadirachtim	Oil antifeedant Miticide	Debug turbo	-0.079	ab	0.046
4	Potassium salts	Organic soap insecticide	Des-X	-0.098	b	0.044
5	*I.fumosorosea* Apopka	Organic microbial insecticide	PFR-97	0.020	ab	0.010
6	Potassium silicate	Organic insecticide / miticide	Sil-matrix	0.013	ab	0.029
7	Sulfur	Contact poison	Sulfur	0.030	a	0.016

[y] Means within each column followed by the same letter are not different from each other at P = 0.05.
[z] SE, standard error.

Greenhouse Seed Treatment Experiment

The use of thiamethoxam seed treatment had no efficacy on WCM populations compared to the untreated control at both dosages tested (Table 2.11, p=0.86). There was no interaction between trial and treatment (p=0.91).

Table 2.11. The effect of thiamethoxam seed treatment on population growth rate of WCM

#	Active ingredient	Mode of action/ Description	Trade name	AI amount	Average population growth rate[y]	SE[z]
1	Control	NA	NA	Control	0.070 a	0.022
2	Thiamethoxam	Neonicotinoid (4A)	Cruiser Maxx	272 g a.i./ha	0.062 a	0.017
	Difenoconazole	Fungicide		327 g a.i./ha		
	Mefenoxam	Fungicide		54 g a.i./ha		
3	Thiamethoxam	Neonicotinoid (4A)	Cruiser Maxx	205 g a.i./ha	0.064 a	0.022
	Difenoconazole	Fungicide		246 g a.i./ha		
	Mefenoxam	Fungicide		41 g a.i./ha		

[y] Means within each column followed by the same letter are not different from each other at P = 0.05.
[z] SE, standard error.

Spring Wheat Field Trial 2013

For the field trial conducted on spring wheat in 2013, untreated control plots had very few mites per plot and moderate WSMV infection (Table 2.12). Average number of mites per leaves sampled in each plot did not differ between treatments. There were no differences between treatments for presence of WSMV (p=0.52). Estimates of yield did not differ between treatments (p=0.77, Table 2.13).

Table 2.12. Average number of wheat curl mites per plot and incidence of *Wheat streak mosaic virus* (WSMV) in spring wheat (*Triticum aestivum* L. cv. Choteau) 2013 field plots.

#	Active ingredient	Mode of action / description	Average WCM per plot post-treatment[x]		SE[y]	Proportion WSMV[xz]		SE[y]
1	Control	NA	2.71	a	0.84	0.50	a	0.15
2	Dimethoate	Organophosphate (1B)	3.57	a	0.97	0.54	a	0.13
3	Chlorpyrifos	Organophosphate (1B)	1.86	a	0.74	0.53	a	0.10
4	Zeta-cypermethrin* S-Cyano methyl	Pyrethroid (3A)	3.43	a	0.78	0.43	a	0.13
5	Lambda-cyhalothrin	Pyrethroid (3A)	1.71	a	0.64	0.61	a	0.10
6	Hexythiazox	Mite growth inhibitor (10A)	2.29	a	1.06	0.52	a	0.15
7	Neem oil	Oil antifeedant	3.00	a	1.07	0.69	a	0.11
8	Azadirachtim *I.fumosorosea* Apopka	Miticide Organic microbial insecticide	2.57	a	0.72	0.39	a	0.14
9	Potassium salts	Organic soap insecticide	4.57	a	1.59	0.62	a	0.13
10	Sulfur	Contact poison	3.57	a	1.13	0.59	a	0.13

[x] Means within each column followed by the same letter are not significantly different from each other at P = 0.05

[y] SE, standard error.

[z] Average proportion of leaves (n=25) per plot infected with WSMV determined by ELISA

Table 2.13. Effect of chemical treatment on yield estimate (kg/ha) in spring wheat (*Triticum aestivum* L. cv. Choteau) 2013 field plots

#	Active ingredient	Mode of action / description	Trade name	Yield (kg/ha)[y]		SE[z]
1	Control	NA	NA	1498	a	125
2	Dimethoate	Organophosphate (1B)	Dimethoate 400	1568	a	194
3	Chlorpyrifos	Organophosphate (1B)	Lorsban 4E	1802	a	279
4	Zeta-cypermethrin* S-Cyano methyl	Pyrethroid (3A)	Mustang Max	1729	a	206
5	Lambda-cyhalothrin	Pyrethroid (3A)	Warrior II	1640	a	253
6	Hexythiazox	Mite growth inhibitor (10A)	Onager	1676	a	180
7	Neem oil Azadirachtim	Oil antifeedant Miticide	Debug turbo	1454	a	114
8	*I.fumosorosea* Apopka	Organic microbial insecticide	PFR-97	1863	a	187
9	Potassium salts	Organic soap insecticide	Des-X	1600	a	150
10	Sulfur	Contact poison	Sulfur	1432	a	163

[y] Means within each column followed by the same letter are not significantly different from each other at P = 0.05
[z] SE, standard error.

Winter Wheat Field Trial 2014

In this trial, we were unable to see a treatment effect due to low presence of WCM and WSMV in control plots. Average number of WCM per plots did not differ between treatments (p=0.78), with many plots having no detectable WCM. WSMV incidence was very low, and did not differ between treatments (p=0.59, Table 2.14). Control plots had 3% of leaves tested infected with WSMV, and highest incidence was 6% leaves infected for plots treated with chlorpyrifos.

Table 2.14. Average number of wheat curl mites per plot and incidence of *Wheat streak mosaic virus* (WSMV) in winter wheat (*Triticum aestivum* L. cv. Genou) 2013-2014 field plots.

#	Active ingredient	Mode of action / description	Average WCM per plot post-treatment[x]		SE[y]	Proportion WSMV[xz]		SE[y]
1	Control	NA	0.71	a	0.84	0.03	a	0.03
2	Methomyl	Carbamate (1A)	0.14	a	0.74	0.01	a	0.01
3	Dimethoate	Organophosphate (1B)	0.43	a	0.97	0.01	a	0.01
4	Chlorpyrifos	Organophosphate (1B)	0.00	a	0.78	0.06	a	0.03
5	Zeta-cypermethrin* S-Cyano methyl	Pyrethroid (3A)	2.71	a	1.06	0.01	a	0.01
6	Lambda-cyhalothrin	Pyrethroid (3A)	3.00	a	1.13	0.03	a	0.02
7	Hexythiazox	Mite growth inhibitor (10A)	1.00	a	0.72	0.01	a	0.01
8	Etoxazole	Mite growth inhibitor (10A)	1.29	a	0.64	0.02	a	0.01
9	Neem oil	Oil antifeedant	0.00	a	1.07	0.03	a	0.01
10	Azadirachtim Potassium salts	Miticide Organic soap	2.00	a	1.59	0.02	a	0.01

[x] Means within each column followed by the same letter are not significantly different from each other at P = 0.05

[y] SE, standard error.

[z] Average proportion of leaves (n=30) per plot infected with WSMV determined by ELISA

No evidence of crop injury was reported, as yield estimates did not differ between chemical treatments and untreated control plots (p=0.28) (Table 2.15).

Table 2.15. Effect of chemical treatment on yield (kg/ha) in winter wheat (*Triticum aestivum* L. cv. Genou) 2013-2014 field plots

#	Active ingredient	Mode of action / description	Trade name	Yield (kg/ha)[y]		SE[z]
1	Control	NA	NA	3033	a	506
2	Methomyl	Carbamate (1A)	Lannate LV	3402	a	467
3	Dimethoate	Organophosphate (1B)	Dimethoate 400	2106	a	113
4	Chlorpyrifos	Organophosphate (1B)	Lorsban 4E	2050	a	511
5	Zeta-cypermethrin* S-Cyano methyl	Pyrethroid (3A)	Mustang Max	2799	a	639
6	Lambda-cyhalothrin	Pyrethroid (3A)	Warrior II	2441	a	466
7	Hexythiazox	Mite growth inhibitor (10A)	Onager	1904	a	224
8	Etoxazole	Mite growth inhibitor (10A)	Zeal	1954	a	244
9	Neem oil Azadirachtim	Oil antifeedant Miticide	Debug turbo	2443	a	653
10	Potassium salts	Organic soap	Des-X	2597	a	538

[y] Means within each column followed by the same letter are not significantly different from each other at P = 0.05.
[z] SE, standard error.

Economic Analysis of Chemical Controls

Insecticide cost is dependent on price per liter, number of sprays conducted per growing season, labor costs and equipment costs. As an example, active ingredient chlorpyrifos costs an average of $12.17 US/L in Montana and North Dakota, based on local pricing when bought in bulk. Recommended maximum spray amount is 526 g a.i./ha, or 1.17 L per hectare, dependent on formulation. Labeled application frequency for wheat is 1-2 sprays per growing season. Cost to spray chlorpyrifos is approximately $14.24 US/ha per application, without including for labor and equipment costs. For chlorpyrifos sprays to be worthwhile on fields infected with WSMV, they need to provide

a yield benefit that covers cost to spray. For a grain price of $150/metric ton ($4/bu) and yields of 5.38 metric ton/ha (approximately 80 bu/ac), Profit/ha=Revenue-Cost =$807-$14.24 =$792.76, when no other input costs are considered. To cover the chemical cost of $14.24/ha, spraying needs to provide a yield benefit of approximately 95kg/ha, 0.51% of 5.38 metric ton/ha. This value underestimates the cost to spray, with labor and equipment costs requiring assessment.

Discussion

Crop viruses can have serious impacts on crop yields and virus control is a challenge. While limiting vector abundance can reduce virus risk there are other factors to consider that may impact the efficacy and sustainability of the practice. Complications include concerns about environmental effects, cost, vector resistance (Castle et al., 2009, Mouchet, 1988), removal of beneficial organisms (Nash et al., 2008) and severity of infection. Yield loss in agricultural systems is influenced by the timing of infection with diseases (Madden et al., 2000) and the timing of pesticide application can be critical in controlling eriophyid mites (Grasswitz, 2012). It is thus important to consider the holistic system, and evaluate the risk-reward ratio to pesticide application and other management options (Hughes et al., 1999). The use of pesticides can be helpful in an integrated management approach, when effective, affordable, and not destructive on the environment. One stage in this process is determining the efficacy of treatments on the vector in question.

In an effort to discover effective acaricides for WCM control, we tested a variety of treatments with varying modes of action. In the greenhouse, we observed consistent efficacy against WCM after application of the carbamate aldicarb and the organophosphate chlorpyrifos. Treatment with sulfur did not reduce WCM populations relative to the untreated control, and is not recommended for use. Mite populations in the 2013 spring wheat field trial did not differ across treatments, and we were precluded from seeing a treatment effect in the 2014 winter wheat field trial due to low disease incidence. Yield (kg/ha) did not differ between treatments for both the spring wheat and winter wheat trials, therefore it is unlikely that treatments caused a phytotoxicity effect on plants. Proportion of plots infected with WSMV did not differ between chlorpyrifos plots and untreated control plots. Further testing is required under visible disease pressure, to evaluate if chemical treatment is economically viable. Disease pressure in the winter wheat trial was very low, therefore no differences could be observed between treatments for WCM and WSMV presence. This difference in infection rate between studies is likely not a variety selection issue, as winter wheat cultivar Genou had 60-65% infection when tested under spring mechanical transmission in Montana (Miller et al., 2014).

Incorporating chemical control as one facet of disease management may decrease the yield loss in farms from WSMV when combined with cultural management including removing the green bridge (Thomas and Hein, 2003), altering planting date to avoid the vector (Staples and Allington, 1956, Hunger et al., 1992), and variety tolerance or resistance (Conner et al., 1991, Harvey et al., 1999, Martin et al., 1984, Seifers et al., 2007). The high rate of reproduction and generational turnover of the WCM (Somsen and

Sill, 1970) may lead to surviving mites after treatment, and subsequent treatments may therefore be required, but with low-cost synthetics, such as chlorpyrifos at approximately $14.24US/ha per application, plus labor and equipment costs, this may be justifiable with further research to that effect. An understanding of the potential of chlorpyrifos as an additional combative tool against WSMV and the clarity that sulfur is not effective at reducing WCM populations is useful information in the search for solutions and information concerning WSMV control in the Great Plains and other wheat growing regions of the world.

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GRASSES AS RESERVOIRS FOR THE WHEAT CURL MITE AND WHEAT STREAK MOSAIC VIRUS

Abstract

The wheat curl mite (WCM, *Aceria tosichella* Keifer) is a generalist pathogen, surviving on a number of grass species and transmitting the damaging *Wheat streak mosaic virus* (WSMV). Grassy disease reservoirs maintain WCM and WSMV between growing seasons. Here, we examined life history traits as they relate to disease reservoir potential. These traits include native status (native or introduced) in the US and life span (annual or perennial) of plant species from different grass tribes. Grasses assessed for their capacity to serve as a disease reservoir included introduced annuals, (*Avena sativa, Lolium perenne multiflorum, Aegilops cylindrica, Triticum aestivum, Setaria italic*) introduced perennials, (*Cortaderia selloana, Festuca rubra, Psathyrostachys juncea, Agropyron cristatum, Pennisetum glaucum*) native annuals, (*Sphenopholis obtusata., Beckmannia syzigachne, Bromus carinatus, Bouteloua curtipendula*, *Echinochloa walteri*) and native perennials (*Schizachyrium scoparium, Poa palustris, Elymus Canadensis, Bromus ciliates*, *Panicum virgatum*). Ability to host WCM ($p=0.011$) and WSMV ($p<0.001$) differed between growth strategies (Annual versus perennial) but did not differ for native status (introduced versus perennial) ($p=0.096$ and $p=0.735$, respectively). All annual plant species served as replicative hosts for WCM. WSMV presence was detected on annual species *Lolium perenne multiflorum*, *Triticum aestivum*

and *Bromus carinatus*. This screening suggests annual grass species are more likely to be WCM-WSMV disease reservoirs than perennial species.

Introduction

WSMV and WCM require a living host for survival (Somsen and Sill, 1970) and can persist in cereal cropping systems as mites move from a maturing host or grassy weeds to a newly emerged crop , a phenomenon known as 'the green bridge.' Eriophyid mites are typically host specific (Oldfield and Proeseler, 1996), however the WCM is unusual as it can survive on at least 90 alternative, non-crop host species (Amrine and Stasny, 1994, Sabelis and Bruin, 1996, Navia et al., 2013). To be an optimal reservoir for disease, plants must support vector reproduction and growth, be susceptible to viral infection, and have the ability to support the replication of transmissible virus (Cronin et al., 2010); qualities influenced by plant resistance, plant tolerance, and vector preference (when applicable) (McElhany et al., 1995, Berger, 1977).

Emigration from wheat of WCM occurs more frequently with a large mite density (Thomas and Hein, 2003) and the same is likely true from alternative hosts. Population growth rate of mites is important in this spread, but impact on disease risk is conditional on mite acquisition of WSMV from alternative hosts. If mites are aviruliferous, yield loss in wheat is minor, caused only by WCM feeding (Harvey et al., 2000, Harvey et al., 2002). These plants are not completely unimportant as reservoirs, as mite populations may later move to WSMV infected plants and become viruliferous, providing a source of initial inoculum.

Removal of disease reservoirs is one control method that can reduce disease. For pests that have potential to move to neighboring farms, area-wide control has been shown to be more effective than localized control (Vreysen et al., 2007). However, where large-scale host eradication is not an option, a similar but less drastic concept is area-wide suppression by reducing density of host plants in a given area (Myers et al., 2000). Suppression of volunteer wheat is a major cultural control method for WSMV (Jiang et al., 2005, Thomas et al., 2004), and suppression of other alternative hosts may also produce positive results. In south-west Australia, experiments conducted in fields after a WSMV epidemic showed virus presence in wheat crops when volunteer wheat and grass species were not removed from the surrounding area, and no disease when grasses were removed in subsequent experiments (Coutts et al., 2008).

Ninety to 100 plant species have been characterized as WCM and WSMV hosts from the Poaceae family (Sill and Connin, 1953, Sill and Agusiobo, 1955, Connin, 1956, Ito, 2011, Ito et al., 2012). Broader classifications of traits that make good WCM-WSMV reservoirs would be useful to predict which alternative host species to target in eradication efforts as discovery of WSMV continues in additional geographic areas. From previous work in other pathosystems, attributes contributing to disease reservoir potential were host lifespan, geographic origin, and phylogeny, with introduced annuals optimal disease reservoirs for the aphid-transmitted BYDV (Cronin et al., 2010), a *Luteovirus*.

Previous studies including surveys of local areas and states, greenhouse experiments and field trials reveal that certain grass tribes are better WSMV and WCM hosts than others (Table 3.01). It is important to note that susceptibility to infection with

WSMV was tested using mechanical inoculation in a number of the papers analyzed, which is less efficient and less reflective of field transmission than disease spread with WCM (Ito et al., 2012). Grass tribes that contain a high proportion of WCM hosts and WSMV hosts are Bromeae, Eragrostideae, Paniceae and Triticeae (Table 3.01). Tribes with a moderate proportion of WCM hosts and WSMV hosts are Andropogoneae, Aveneae, Cynodonteae and Poeae (Table 3.01). Tribes Aristideae, Danthonieae, Meliceae, Oryzeae Stipeae and Zoysieae have not been adequately assessed for proportion of species hosting the WCM and WSMV in previous work (Table 3.01).

Table 3.01. Proportion of species analyzed from various grass tribes hosting wheat curl mites (WCM) and *Wheat streak mosaic virus* (WSMV), a literature review.

Grass Tribe	WCM hosts[x]	WSMV hosts[y]	Number of plant species assessed for WCM[z]	Number of plant species assessed for WSMV[z]
Andropogoneae	0.43	0.20	7	15
Aristideae	0.00	NA	1	0
Aveneae	0.40	0.50	5	4
Bromeae	1.00	0.67	5	6
Cynodonteae	0.67	0.43	9	7
Danthonieae	0.00	0.00	1	1
Eragrostideae	0.60	0.75	5	4
Meliceae	0.00	NA	1	0
Oryzeae	0.00	NA	1	0
Paniceae	0.86	0.82	14	17
Poeae	0.50	0.29	12	7
Stipeae	1.00	NA	2	0
Triticeae	0.82	0.50	17	16
Zoysieae	1.00	1.00	1	3

[x] Proportion of species tested infested with WCM.
[y] Proportion of species tested infected with WSMV.
[z] Data from (Christian and Willis, 1993, Sill and Agusiobo, 1955, Connin, 1956, Somsen and Sill, 1970, Sill and Connin, 1953, Coutts et al., 2008, Carew et al., 2009, Seifers et al., 2010).

The study completed in this manuscript analyzed twenty grass species for susceptibility to WCM and WSMV under viruliferous WCM infestation. Classification of grass species were life history traits including annuals vs. perennials, representatives were chosen from dissimilar grass tribes, and both native grasses and those introduced to the US were evaluated. This study tested those twenty grass species for 1) the ability to host WCM populations 2) the ability to become infected with WSMV, and 3) competence to serve as a WSMV acquisition host for WCM.

Materials and Methods

Mite Population and Virus Isolate

Mites used in greenhouse experiments were sourced from a colony established from WCM collected in September 2007 from the Arthur H. Post Agronomy Research Farm, Bozeman MT. The colony was maintained in a plant growth chamber at 18-24°C as described by Ito *et al*, 2012. The population was fortified with WCM collected from Gallatin County, MT, to keep it representative of the local WCM population. Mite populations were originally grown on a mixed population of susceptible spring wheat cultivars: Amidon, Fortuna and Choteau, and later only on Choteau, in MSU mix soil (described above). Every few months, wheat plants of the WCM population were re-inoculated with WSMV isolate Conrad-I (stored at -80°C), originally collected in 2007 from symptomatic winter wheat in Conrad, MT (Ito et al., 2012). To mechanically transmit the virus to the wheat plants, a 1:10 mixture of macerated infected leaf tissue and phosphate buffered saline (PBS; pH 7.2, 136.9mM NaCl, 8.1mM Na_2HPO_4, 1.5 mM of

KH_2PO_4 and mM KCl), plus 1% of carborundum (320 grit) to abrade the leaf surface, were combined in a plastic extraction bag (Agdia, Inc., Elkhart, IN). Using gloved fingertips, the virus mixture was gently rubbed onto wheat leaves. Source plant leaves were tested for WSMV using ELISA (described above) 2-3 weeks after inoculation to confirm WCM populations were viruliferous.

Species Selection

Species selected were grasses occurring in the Great Plains region of the United States, with representatives of interest chosen from introduced annuals, native annuals, introduced perennials and native perennials (Table 3.02). Data on plant location, native status, and duration (life span) was obtained from the USDA Natural Resources Conservation Service (NRCS) Plants Database (NRCS, 2015). Analysis of previous studies facilitated plant selection from various grass tribes, including surveys of local areas and states, greenhouse experiments and field trials (Christian and Willis, 1993, Sill and Agusiobo, 1955, Connin, 1956, Somsen and Sill, 1970, Coutts et al., 2008, Carew et al., 2009, Seifers et al., 2010, Ito et al., 2012), as some tribes are known to have more hosts species for WCM and WSMV, and other tribes typically are non-hosts for the disease.

Table 3.02. Grass species selection and their native status and life span

Scientific name	US Native Status[y]	Life span[z]	Common name	Grass tribe
Avena sativa L.	I	A	Common oat	Aveneae
Lolium perenne L. subsp. *multiflorum* (Lam.) Husnot	I	A	Annual ryegrass	Poeae
Aegilops cylindrica Host	I	A	Jointed goatgrass	Triticeae
Triticum aestivum L.	I	A	Spring wheat	Triticeae
Setaria italica (L.) P. Beauv.	I	A	Foxtail millet	Paniceae
Sphenopholis obtusata (Michx.) Scribn.	N	A	Prairie wedgegrass	Aveneae
Beckmannia syzigachne (steud.) Fernald	N	A	Slough grass	Aveneae
Bromus carinatus Hook. & Arn.	N	A	California brome	Bromeae
Echinochloa walteri (Pursh) A. Heller	N	A	Coast cockspur	Paniceae
Cortaderia selloana (Schult. & Schult. F.) Asch. & Graebn.	I	P	White pampas grass	Danthonieae
Festuca rubra L. subsp. *fallax* (Thuill.) Nyman	I	P	Chewings fescue	Poeae
Psathyrostachys juncea (Fisch.) Nevski	I	P	Russian wildrye	Triticeae
Agropyron cristatum (L.) Gaertn.	I	P	Crested wheatgrass	Triticeae
Pennisetum glaucum (L.) R. Br.	I	P	Pearl /Purple millet	Paniceae
Bouteloua curtipendula (Michx.) Torr.	N	P	Sideoats grama	Cynodonteae
Schizachyrium scoparium (Michx.) Nash	N	P	Little blue stem	Andropogoneae
Poa palustris L.	N	P	Fowl bluegrass	Poeae
Elymus canadensis L.	N	P	Canada wildrye	Triticeae
Bromus ciliatus L.	N	P	Fringed brome	Bromeae
Panicum virgatum L.	N	P	Switch grass	Paniceae

[y] I = introduced to the United States and N = native to the United States.

[z] A = annual species and P = perennial species.

Susceptibility of Grasses to WCM and WSMV

Viral incidence and mite absence or presence was determined from six replications, with one pot of five plants considered a replication. Two additional pots of each species were grown under the same conditions and at the same time as a negative control, and not infested, for ELISA testing. Seeds were planted ten per pot at an approximate depth of 2 cm in MSU mix soil (1:1:1 Canadian sphagnum peat moss, mineral soil mix and Aquagro 2000G [Aquatrols, NJ]) with greenhouse conditions 16h day: 8h night at 24 ± 4°C and 18 ± 4°C, respectively. Pots were thinned at the seedling stage. Design was completely randomized design in the greenhouse of the Plant Growth Center, Montana State University, Bozeman MT. Plants were inoculated with viruliferous mites using the leaf piece method when plants were big enough to sustain the leaf piece (2-3 leaf) by attaching leaves 3-5 cm in length holding 10 WCM to the experimental units with paper clips to the adaxial surface of leaves. Leaf pieces were attached close to the base of stems on the youngest expanded leaf. The number of WCM on each leaf piece was recorded. After two days, leaf pieces were removed and the number of WCM counted on the leaf piece to get an estimate of the number of WCM transferred to the experimental plant.

Thirty days post-inoculation, plants were checked for the presence of WCM using a dissecting microscope at 10x magnification, checking the adaxial surface of all leaves from each plant. Four to six leaves from each plant were enclosed in 10.2 X 15.2 cm sample bags (Fisher Scientific, Pittsburgh, PA) and frozen at -20°C for testing for WSMV with ELISA.

Wheat Curl Mite Competence

This experiment was performed to compare the ability of introduced, native, annual and perennial grasses found throughout the Great Plains to determine the reservoir potential of hosts by analyzing if WCM acquire WSMV from the various grasses during feeding and transmit the disease to directly neighboring wheat plants. An aviruliferous WCM colony was grown on a mixed population of susceptible spring wheat cultivars: Amidon, Fortuna, and Choteau. Pots measuring 12.7cm in diameter were filled with the soil media MSU mix (1:1:1 Canadian sphagnum peat moss, mineral soil mix and Aquagro 2000G [Aquatrols, NJ]). Eight plants were grown in the pots until they were at growth stage DC 14-15 (4-5 leaves unfolded) (Zadoks et al., 1974), before inoculating with 20-30 WCM eggs per plant using a toothpick with an eyelash hair attached. Eggs were used to insure the virus free nature of the sample. This population was maintained in the growth chamber at 18-24°C and plants were enclosed in a plastic cover (14 cm height, 8.5 cm diameter, Pro-Kal containers, Michigan) with three window cut-outs (5cm x 2cm) sealed with a fine nylon lab pak mesh (25µm; Sefer AG, Switzerland) to avoid contamination. Seeds of the 20 grass species were planted six per pot (12.7cm diameter pots 9 cm height) at approximate depth of 2 cm in MSU mix soil (1:1:1 Canadian sphagnum peat moss, mineral soil mix and Aquagro 2000G [Aquatrols, NJ]) with greenhouse conditions 16h day: 8h night at 24 ± 4°C and 18 ± 4°C, respectively. Pots were thinned to one plant per pot, when plants were seedlings. Planting of species was staggered, with species taking longer to grow planted first, to insure roughly uniform growth stages of species. Preliminary tests for germination and growth time were

performed prior and recorded. The pots were arranged as a completely random design in the greenhouse of the Plant Growth Center, Montana State University, Bozeman MT with six replications, with one pot for each replication.

Plants were mechanically inoculated with WSMV Conrad-I isolate, using frozen infected tissue in a 1:10 ratio with PBS when plants were at least at the four leaf stage. Infected plant tissue was macerated in a KitchenAid food processor diluted 1:10 (wt:vol) with PBS and 1% (wt/vol) carborundum was added to make virus inoculum, and stored on ice. Gloved hands were used to gently rub the solution into every leaf of the experimental plant populations, using thumb and forefinger. Plants were infested with aviruliferous mites using the leaf piece method seven days after WSMV mechanical inoculation attaching leaves 3-5 cm in length holding 10 WCM to the experimental units with paper clips to the adaxial surface of leaves. Leaf pieces were attached close to the base of stems on the youngest expanded leaf of plants. The number of WCM on each leaf piece was recorded. After two days, leaf pieces were removed. Three seeds of spring wheat (*Triticum aestivum* L. cv. Choteau) were planted at a depth of approximately 2 cm, 1 cm away from the base of experimental plants, and thinned to two plants at growth stage DC 13 (3 leaves unfolded) (Zadoks et al., 1974). After 25 d, wheat plants were checked for the presence of WCM using a dissecting microscope at 10x magnification, by checking the adaxial surface of all leaves. Collection of samples for ELISA testing for WSMV presence was undertaken; six leaves from each wheat plant and each alternative host stored in 10.2 X 15.2cm sample bags (Fisher Scientific, Pittsburgh, PA) and frozen at -20°C.

Statistical Analysis

Data was analyzed using R software, version 3.1.2. Percent incidence data of WSMV was logit transformed and analyzed using a linear mixed-effect model with origin and lifespan as fixed effects and replicate as a random effect. WCM number per plant was analyzed using a linear mixed-effect model with origin and lifespan as fixed effects and replicate as a random effect. The assumption of homogeneity of variances was tested using Levene's test. Data displayed are non-transformed values, and WCM data displayed is presence absence.

Results

Susceptibility of Grasses to WCM and WSMV

Grassy species from the Great Plains were tested for their susceptibility to WSMV, ability to host WCM populations and ability to infect aviruliferous WCM with WSMV and spread disease and vector to neighboring wheat plants. Incidence of WSMV differed between the grass species ($p<0.001$), as did ability to host WCM populations ($p=0.015$). WSMV incidence differed between annual species and perennial species ($p<0.001$), and a marginal difference was reported between native species and introduced species ($p=0.096$). Number of WCM per plant differed between annual species and perennial species ($p=0.011$), but not between native species and introduced species ($p=0.735$). Only two introduced annual species, *L. perenne* subsp. *multiflorum* and *T. aestivum*, became infected with WSMV with 16% and 67% plants, respectively (Table

3.03). Introduced annual species tested had at least 33% of plants infested with WCM, with *T. aestivum* and *A. cylindrica* having all plants infested with WCM (Table 3.03).

Table 3.03. Detection of *Wheat streak mosaic virus* (WSMV) and wheat curl mites on grass species inoculated with viruliferous WCM under greenhouse conditions.

Plant	Grass tribe	Origin / Growth[w]	Proportion plants infected with WSMV[x]	SE[y]	Proportion plants hosting WCM[z]	SE[y]
A. sativa	Aveneae	IA	0.00	0.00	0.33	0.38
L. perenne multiflorum	Poeae	IA	0.17	0.34	0.50	0.35
A. cylindrica	Triticeae	IA	0.00	0.00	1.00	0.00
T. aestivum	Triticeae	IA	0.67	0.27	1.00	0.00
S. italica	Paniceae	IA	0.00	0.00	0.33	0.38
S. obtusata	Aveneae	NA	0.00	0.00	0.67	0.27
B. syzigachne	Aveneae	NA	0.00	0.00	1.00	0.00
B. carinatus	Bromeae	NA	0.17	0.34	1.00	0.00
E. walteri	Paniceae	NA	0.00	0.00	0.50	0.35
C. selloana	Danthonieae	IP	0.00	0.00	0.67	0.27
F. rubra	Poeae	IP	0.00	0.00	0.00	0.00
P. juncea	Triticeae	IP	0.00	0.00	0.00	0.00
A. cristatum	Triticeae	IP	0.00	0.00	0.17	0.34
P. glaucum	Paniceae	IP	0.33	0.38	0.83	0.15
B. curtipendula	Cynodonteae	NP	0.00	0.00	0.67	0.27
S. scoparium	Andropogoneae	NP	0.00	0.00	0.17	0.34
P. palustris	Poeae	NP	0.00	0.00	0.33	0.38
E. canadensis.	Triticeae	NP	0.67	0.27	0.00	0.00
B. ciliatus	Bromeae	NP	0.33	0.38	0.67	0.27
P. virgatum	Paniceae	NP	0.00	0.00	0.17	0.34

[w] I= Introduced species, N=Native species, A=Annual species, P=Perennial species
[x] Proportion of plants ELISA-positive for WSMV
[y] Standard error of the mean
[z] Proportion of plants infested with WCM

Bromus carinatus was the only native annual species tested susceptible to WSMV after viruliferous mite inoculation. The only introduced perennial species testing positive for WSMV was *P. glaucum* (Table 3.03). WSMV was detected with ELISA on two native perennial species, *E. canadensis* and *B. ciliatus*, however WCM were not found on

E. canadensis, suggesting they are not good replicative hosts for WCM (but WCM survived long enough to infect plants) but can be infected with WSMV (Table 3.03). *F. rubra* and *P. juncea* did not support WCM populations and WSMV presence was not detected in leaf tissue.

Wheat Curl Mite Competence

Plant species that did not host WCM or test positive for WSMV (Table 3.03) were excluded from analysis for ability to infest and infect neighboring wheat plants, as they were not hosts themselves. Spread of WCM and WSMV to neighboring wheat plants occurred with *T. aestivum* (introduced annual) and *P. glaucum* (introduced perennial) (Table 3.04).

Table 3.04. Transmission of WCM and WSMV from grassy hosts to neighboring wheat plants.

Plant	Grass tribe	Origin / Growth[w]	Proportion wheat plants infested with WCM[x]	SE[y]	Proportion wheat plants infected with WSMV[z]	SE[y]
L. perenne multiflorum	Poeae	IA	0.00	0.00	0.00	0.00
T. aestivum	Triticeae	IA	0.33	0.38	0.08	0.26
B. carinatus	Bromeae	NA	0.17	0.34	0.00	0.00
P. glaucum	Paniceae	IP	0.08	0.26	0.08	0.26
B. ciliatus	Bromeae	NP	0.25	0.38	0.00	0.00

[w] I= Introduced species, N=Native species, A=Annual species, P=Perennial species
[x] Proportion of wheat plants found to have at least one living WCM
[y] Standard error of the mean
[z] Number of wheat plants ELISA-positive for WSMV

Wheat plants grown in contact with *B. carinatus* (native annual) and *B. ciliatus* (native perennial) harbored WCM on 17% and 25% of wheat plants, respectively, but did not test

positive for WSMV. Wheat plants in contact with *L. perenne* multiflorum plants were not infested with WCM after inspection under the microscope and did not test positive for virus (Table 3.04).

Discussion

Understanding the impacts of provenance and life span on performance of species as disease reservoirs can reveal potential risks of disease development in wheat systems and facilitate disease management. In other pathosystems, for example *Barley yellow dwarf virus* (BYDV), short-lived introduced species were good disease reservoirs, due to high plant metabolism and poorly defended tissue (Cronin et al., 2010). For the 20 grass species tested in this study, growth cycle (annual versus perennial) had the biggest impact on ability of grasses to host WCM and susceptibility of grasses to WSMV. Ability to host mites and virus did not differ between introduced and native plants, indicating that this trait is less important for disease reservoir studies of WCM and WSMV. All annual species tested herein served as reproductive hosts of WCM. The optimal reproductive WCM hosts were *A. cylindrica*, a common weed species in the US, wheat *T. aestivum* a grain crop grown globally, *B. syzigachne*, a species typically located close to wetlands and *B. carinatus*, a forage grass and weed. Introduced perennial species *C. selloana*, a weed in some systems and a forage, and *P. glaucum*, a crop, were also replicative WCM hosts. *Agropyron cristatum*, a forage grass in the US, had WCM present on 17% plants. Introduced perennials that did not support WCM in this study were *F. rubra*, a grass used in turf applications, and *P. juncea*, a forage. Native perennial species serving as WCM

hosts were *S. scoparium*, *P. palustris*, *B. ciliatus* and *P. virgatum*; whilst no WCM were found on *E. canadensis* plants. Interestingly, WSMV was detected on *E. canadensis* plants but WCM were not found. This is likely due to WCM being present but challenging to find, or WCM populations did not survive for the length of the experiment and plants were not sufficient mite hosts. Difficulty arose analyzing thin-leaved plants for WCM such as *B. syzigachne, B. curtipendula*, *C. selloana* and *S. scoparium*.

Plants that tested positive for WSMV were *L. perenne* multiflorum, *T. aestivum*, *B. carinatus*, *P. glaucum*, *E. canadensis* and *B. ciliatus.* For grasses to be disease reservoirs, ability to host WCM and WSMV is crucial, and another critical aspect is whether the plant can spread virus to new generations of WCM during feeding, as WSMV does not spread transovarially, and WCM reproduce rapidly. Aviruliferous WCM were transferred to the experimental grass species after mechanical inoculation with WSMV, to test whether feeding mites acquired the virus and subsequently transmitted the virus to neighboring wheat plants. The results of this preliminary study revealed that four grasses spread WCM to neighboring wheat plants, and two grasses spread both WCM and WSMV to the neighboring wheat. The limitation of this study is that WCM transfer from experimental plants to directly neighboring wheat via direct plant contact is not representative of mite movement in the field, which is primarily wind-driven (Slykhuis, 1955, Staples and Allington, 1956). Subsequent studies focusing on greenhouse wind tunnels (Thomas and Hein, 2003), or field experiments with bordering strips of grassy hosts infested with aviruliferous mites and mechanically inoculated with virus would be a way to overcome the shortcomings of this greenhouse experiment.

This study indicates that growth strategy impacts grass species ability to host WCM and WSMV, with annual plants the worst culprit for providing disease reservoirs and likely for perpetuating disease in wheat systems. For wheat systems, locating WCM source populations should begin with assessing annual species (Ellis et al., 2004), particularly weeds, and planning for cultural control actions, with consideration of mite movement via wind and proximity of plants in the system to crops.

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CONCLUSION

In summary, greenhouse populations of the wheat curl mite (WCM), vector to *Wheat streak mosaic virus* (WSMV), were reduced under chemical treatment with active ingredient chlorpyrifos ($p<0.001$). This is the only treatment currently available for use in the Great Plains that has shown efficacy at reducing WCM populations. Extension of this knowledge to field situations requires further work to rigorously test it and discover optimal chemical application timing and rates, and determine yield benefit estimates. Assessment of 20 grass species from the Great Plains (*A. sativa, L. perenne* multiflorum*, A. cylindrica, T. aestivum, S. italica*, *C. selloana, F. rubra, P. juncea, A. cristatum, P. glaucum, S. obtusata, B. syzigachne, B. carinatus, B. curtipendula*, *E. walteri*, *S. scoparium, P. palustris, E. canadensis, B. ciliates* and *P. virgatum*) for capacity to serve as disease reservoirs for WCM and WSMV revealed that lifespan was greatly influential on disease reservoir potential, both for WCM ($p=0.011$) and WSMV ($p<0.001$). All annual plant species served as replicative hosts for WCM. Annual species *L. perenne*, *T. aestivum*, and *B. carinatus* were infected with WSMV after infestation with viruliferous WCM.

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