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Impact of *Wheat streak mosaic virus* and *Triticum mosaic virus* on transmission by *Aceria tosichella* Keifer (Eriophyidae) and virus epidemiology in wheat

Camila F. de Oliveira  
*University of Nebraska-Lincoln*

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**IMPACT OF *WHEAT STREAK MOSAIC VIRUS* AND *TRITICUM MOSAIC  
VIRUS* ON TRANSMISSION BY *ACERIA TOSICHELLA* KEIFER  
(*ERIOPHYIDAE*) AND VIRUS EPIDEMIOLOGY IN WHEAT**

by

Camila F. de Oliveira

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**IMPACT OF *WHEAT STREAK MOSAIC VIRUS* AND *TRITICUM MOSAIC VIRUS* ON TRANSMISSION BY *ACERIA TOSICHELLA* KEIFER (ERIOPHYIDAE) AND VIRUS EPIDEMIOLOGY IN WHEAT**

**Camila F. de Oliveira, M.S.**

**University of Nebraska, 2013**

**Adviser: Gary L. Hein**

The wheat curl mite (WCM), *Aceria tosichella* Keifer, transmits a complex of viruses, *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV) and *Wheat mosaic virus* (WMoV), to wheat, *Triticum aestivum*, in the Great Plains. Co-infection of wheat by these viruses is frequently observed, increasing disease severity and yield loss.

Current genetic work classifies WCM populations into two genotypes, Type 1 and Type 2. It has been shown that different mite genotypes are able to transmit viruses at varying rates. WCM-virus relations are very specific and can impact vector biology. In this study, the primary objective was to determine if co-infection of wheat by WSMV+ TriMV has an impact on each virus transmission rate by the WCM Type 1 and Type 2. An additional objective was to establish the impact of double viral infections on the biology of the mites and virus dispersal in the field.

Using a series of transmission studies, it was determined that Type 1 WCMs do not transmit TriMV even in the presence of WSMV. Type 2 WCMs feeding on wheat infected with both viruses, have reduced WSMV transmission when compared to mites feeding on singly inoculated plants. However, TriMV transmission is increased when

mites feed on wheat infected by both viruses. WSMV reduction in transmission, might be due to the fact that mites feeding on double infected plants have reduced survival when compared to single inoculated plants.

Mite counts from the field indicated that mites feeding on WSMV infected plants had the highest populations, followed by the control, WSMV+TriMV and TriMV. In field conditions, WSMV transmission by Type 2 WCM was reduced when exposed to source plants with WSMV+TriMV. TriMV transmission was not different between mites feeding on single or double infected plants. Hence, in laboratory conditions, we saw the transmission of TriMV benefitting from co-infection with WSMV in wheat. But in the field, mites feeding on TriMV plants have reduced reproduction, which counters the enhanced transmission found in the laboratory. These findings enhance the understanding of WCM virus complex epidemiology.

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## **Chapter 1: Literature Review**

## Introduction

The wheat curl mite (WCM), *Aceria tosichella* Keifer, is a microscopic mite from the family Eriophyidae that is present worldwide. It is the sole vector of *Wheat streak mosaic virus* (WSMV), *Wheat mosaic virus* (WMoV) and *Triticum mosaic virus* (TriMV) viruses (Slykuis 1955, Seifers *et al.* 1997, Seifers *et al.* 2009). Management of this disease complex is done primarily via vector management.

WSMV is more prevalent than the other viruses; however, co-infection with the other two viruses is common in wheat fields (Burrows *et al.* 2009). TriMV is mostly detected when the plant is co-infected with WSMV (Byamukama *et al.* 2013). Co-infection with TriMV and WSMV worsens disease symptoms in susceptible wheat cultivars (Tatineni *et al.* 2010).

WCM collected from across the Great Plains separate into two main genotypes (Hein *et al.* 2012). These genotypes can both transmit WSMV but differ significantly in the transmission rates of TriMV and WMoV (Seifers *et al.* 2002, McMechan 2012). The impact of double infections on the transmission rates of individual viruses is still unclear. Given that different mite genotypes vary in transmission rates for some viruses, further research on vector mediated virus interactions is needed to better manage this disease complex.

## Impact of WCM Disease Complex

Non-viruliferous WCM infestations have been shown to cause 1-17% yield loss depending on the severity of infestation (Harvey *et al.* 2000). However, the main losses due to WCM are associated with the transmitted viruses. In Kansas, the WSMV disease complex (including TriMV and WMoV) was the second greatest cause of winter wheat

yield loss, causing an average loss of 1.7% in 2011 (Appel *et al.* 2011). In 2012 and 2013, losses were reduced to 1.2%, each year, which is equivalent to 5.4 million bushels of wheat (Appel *et al.* 2012, Appel *et al.* 2013).

WCM infestations in young plants cause total leaf trapping. Infestations in the spring, when the plants are older, cause mild rolling of leaf edges (Staples and Allington 1956). Virus symptoms usually can be seen in the spring when the plants look yellow, rosetted and stunted. WSMV outbreaks are more severe if infection occurs early in the fall and in years with warm temperatures (Wegulo *et al.* 2007).

### **Management of WCM Transmitted Viruses**

The critical epidemiological factor for the wheat-mite-viruses complex is the “green bridge” period; where volunteer wheat and alternate hosts serve as a refuge for WCM outside the winter wheat growing periods, perpetuating the disease cycle until the next fall planting. Volunteer wheat that arises after hailstorms as kernels are shattered to the ground and germinate is the most important green bridge host. Volunteer wheat germinating before harvest poses an increased risk for fall infestations, since mites much more readily move to the new plants (Staples and Allington 1956). Post-harvest volunteer wheat poses a smaller risk than pre-harvest volunteer wheat since mites cannot survive long without a host (i.e. after harvest).

Some annual and perennial grasses, as well as some crops, also serve as alternate hosts to the mite and as reservoirs to the virus (Connin 1956a). Effective chemical control is currently not available for the WCM. Planting tolerant varieties is used as a management tool if the location is under high-risk for the diseases.

## **Cultural control**

Pre-harvest volunteer wheat is the primary over-summering host for WCM. Given that WCM do not survive without a host, volunteer wheat must be controlled at least fourteen days before winter wheat planting to prevent mite dispersal into the new crop (Wegulo *et al.* 2007). Different control methods vary in application time as well as in degrees of effectiveness against the volunteer and its mite populations. Herbicides provide alternative control of volunteer wheat if tillage is not viable. Herbicides such as glyphosate (Monsanto, St. Louis, Missouri) and paraquat (Zeneca Ag. Products, Wilmington, Delaware) can effectively control volunteer wheat and reduce WCM infestation risk if applied well in advance of fall wheat planting (Jiang *et al.* 2005). Paraquat is a contact herbicide and it quickly kills volunteer, greatly reducing mite populations. Glyphosate is a systemic herbicide, slow to kill volunteer, and if used too close to fall planting it will not be effective at rapidly reducing mite presence and spread. However, if used with proper timing, glyphosate might be more viable than paraquat due to lower toxicity and cost (Jiang *et al.* 2005).

Tillage is effective in controlling volunteer wheat, if rapid control of volunteer wheat is necessary (Thomas *et al.* 2004). Tillage can deplete soil moisture, thus, it may not be a practical solution for volunteer wheat control in all situations. Under dry and hot conditions, tillage reduces mite populations more rapidly and efficiently than glyphosate (Thomas *et al.* 2004).

Avoiding early planting of winter wheat can diminish the risk of WCM (Wegulo *et al.* 2007). Warm temperatures in the fall provide ideal conditions for early mite infestation and infection of wheat by viruses (Staples and Allington 1956). WCM move

more with warmer temperatures, hence increasing the likelihood of disease in the field (Nault and Styer 1969).

### **Host plant resistance**

Host plant resistance for managing the WCM virus complex has targeted both vector and virus resistance. Virus resistant wheat lines are currently available that target WSMV. Currently two main sources of WSMV resistance have been incorporated into winter wheat. The *Wsm-1* gene, present in the winter wheat cultivar ‘Mace’ (Graybosch *et al.* 2009), was introgressed to winter wheat from *Thinopyrum intermedium* Barkworth & Dewey (Friebe *et al.* 2009). Under ideal conditions, Mace is also resistant to TriMV (Tatineni *et al.* 2010; Byamukama *et al.* 2012). The second main source of WSMV resistance, *Wsm-2*, came from the CO960293-2 wheat germplasm line. The origin of resistance of CO960293-2 line is unclear since its parents were not reported to be resistant to WSMV (Haley *et al.* 2002, Seifers *et al.* 2006). *Wsm-2* has been incorporated into ‘RonL’ (Seifers *et al.* 2007) and ‘Snowmass’ (Haley *et al.* 2011) winter wheat cultivars. Both *Wsm-1* and *Wsm-2* are temperature sensitive. Mace exhibits mild to moderate symptoms 28 days post inoculation at 20-26 °C (Tatineni *et al.* 2010). *Wsm-2* resistance is effective at 18 °C but is ineffective when temperatures surpass 24°C (Seifers *et al.* 2006). These winter wheat lines provide good levels of resistance if planted when fall temperatures are cooler (Seifers *et al.* 2006).

Plant resistance against the vector acts via antibiosis, reducing mite populations and colonization. The winter wheat cultivar ‘TAM 107’ successfully controlled WCM populations and was grown extensively from the mid 1980’s until the beginning of the 1990’s (Harvey *et al.* 1997). WCM strains overcame the resistance in TAM 107 and other

cultivars, and their use was discouraged (Harvey *et al.* 1995, Harvey *et al.* 1997). A promising wheat line 'OK05312' (Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK) has shown resistance to the WCM in the lab. Mites reproduce less on this line, and the leaf suffers less rolling/folding than WCM-susceptible winter wheat cultivars 'Jagger' and 'Ike'. Further field trials need to be conducted before this variety is made available to growers (Muragan *et al.* 2011).

### **Biology and Ecology of the WCM**

The family Eriophyidae is distinguished by possessing only two pairs of legs. WCM are white and have a vermiform shape and measure about 250  $\mu$  in length (Keifer 1938). Leaf curling is a common symptom of WCM presence and leaves can show curled appearance even with small mite populations. Upon infestation, new leaves curl completely and become trapped in older leaves as they emerge (Staples and Allington 1956). But, infestation on older leaves might only cause leaf rolling on the edges (Staples and Allington 1956).

The complete life cycle from egg-to-egg takes from 7-10 days to complete at 24°C to 27°C (Staples and Allington 1956). There are four life stages of the wheat curl mite; egg, larva, nymph and adult. Eggs hatch after about 72 hours at 25°C, remain as larvae for approximately 36 hours, and then enter a quiescent stage for 18 hours (Staples and Allington 1956). This pattern also holds true from nymph to adult, thus, taking 4-5 days from egg hatch to adult. Females have a pre-oviposition period of one to two days (Staples and Allington 1956). It is not known precisely how long WCM live as adults.

WCM have arrhenotokous parthenogenesis as a mode of reproduction where fertilized eggs develop into females and unfertilized eggs into males (Helle and Wysoki



1983). Thus, an unfertilized female that lands on a new plant will give rise to only male offspring. The female will mate with these males and start a new colony. Eriophyidae mating occurs through indirect sperm transfer. The male deposits the spermatophore on the leaf and the female picks it up (Oldfield *et al.* 1970). Each female can lay at least 12 eggs, but it may average up to 22 (Staples and Allington 1956, Al-Azzazy *et al.* 2013).

High relative humidity is essential for egg hatching. With temperatures of 25°C, eggs readily hatched at relative humidity (RH) of 75 and 100%, but they desiccated if relative humidity was below 75% (Slykhuis 1955). Holding eggs for eight days at 5°C, 15°C and 25°C with 100% RH did not negatively affect egg hatchability (Slykhuis 1955). Eggs are also extremely tolerant to cold conditions, being able to survive up to three days at -20°C, but adult mites can only survive up to two days at -15°C (Slykhuis 1955). In laboratory conditions WCM colonies can be maintained at 5°C, but egg viability will be reduced (Skare *et al.* 2003).

### **Classification and Identification**

*Aceria tulipae* Keifer (Keifer 1938) was first described in tulips imported from Holland, and it was thought to feed on *Allium spp.*, *Liliaceae spp.*, as well as on wheat. *A. tulipae* was later described as the vector of WSMV (Slykhuis 1955), and many publications on vector capabilities used this name (del Rosario and Sill 1965, Slykhuis 1956, Nault and Styer 1970). Given that eriophyid mites are often host-specific, it was odd that *A. tulipae* was present on hosts from different plant genera. Shevtchenko *et al.* (1970) morphologically distinguished *A. tulipae* from onion and wheat as different species. He proposed a new species and described the wheat curl mite from wheat, *Aceria tritici* Shevtchenko. However, previous to Shevtchenko, Keifer (1969) described *Aceria*

*tosichella* from wheat and barley in Yugoslavia. Since Keifer published on *A. tosichella* first, his species name takes precedence. Morphological characteristics used to describe *Aceria* are similar to those used to identify the *Eriophyes* genus. The two genera were then combined under *Eriophyes* (Newkirk and Keifer 1971). In 1989, The International Commission of Zoological Nomenclature reestablished *Aceria* as a distinct genus from *Eriophyes* (Amrine and Stasny 1994). Thus, the WCM has a long history of taxonomic confusion, and it has been described in the literature as *Aceria tulipae*, *Eriophyes tulipae* and *Aceria tosichella*.

The first indication of the existence of host strains of WCM was observed in 1955 as WCM from *Hordeum jubatum* L., *Elymus Canadensis* L. and *Agropyron smithii* L. could not be reared in wheat, and mites from wheat could not be reared in these grasses (Slykhuis 1955). In 1965, del Rosario and Sill reported the occurrence of mite strains adapted to specific hosts and these strains also varied in WSMV transmission. Mites collected from *Agropyron smithii*, but reared in wheat, transmitted WSMV to wheat at a 32% rate, while mites originally from wheat transmitted WSMV at a 84-92% rate (del Rosario and Sill 1965). Harvey *et al.* (1995a) found indications of WCM biotypic differences in the lab as one mite population overcame TAM 107 resistance in 6 weeks and the other population in two months. Additionally, different populations of WCM collected throughout the Midwest and Canada vary in virulence to different sources of resistance in wheat (Harvey *et al.* 1995b, Harvey *et al.* 1999).

Currently there are two main genotypes of WCM discussed in the literature. Using polymerase chain reaction (PCR) to amplify nuclear ribosomal internal transcribed spacer (ITS1), mitochondrial 16S rRNA gene and nuclear ANT gene, Carew *et al.* (2009)

found that mites collected in Australia are from two different lineages, named Type 1 and Type 2. In the U.S., populations collected from South Dakota (SD), Montana (MT), Kansas (KS), and Texas (TX) are a different lineage based on ribosomal and mitochondrial DNA than a Nebraska (NE) population (Hein *et al.* 2012). SD, MT, KS and TX are equivalent to the Type 1 mites from Australia and NE to Type 2 (Hein *et al.* 2012).

These WCM populations also differ in their vector capabilities of WSMV, TriMV and WMoV. The five WCM populations differed in their ability to transmit different WMoV isolates (Seifers *et al.* 2002). Although NE transmitted at higher rates, NE and MT colonies could readily transmit all isolates of WMoV. But, KS only transmitted one WMoV isolate and SD and TX did not transmit WMoV (Seifers *et al.* 2002). In Australia, WSMV is only transmitted by Type 2 mites (Schiffer *et al.* 2009), while in the U.S., WSMV is transmitted by both mite types (Seifers *et al.* 2002). TriMV is transmitted by Type 2 mites and only transmitted at low rates by Type 1 WCM if populations are high (McMechan 2012). The different WCM lineages co-exist in the field and small genetic exchange may be possible as a small sample of WCM had both Type 1 and Type 2 ITS1 profiles (Carew *et al.* 2009).

### **Movement and Distribution of WCM**

WCM feeding behavior dictates mite movement within the plant. They feed on bulliform cells, located in the grooves of veins (Orlob 1966). WCM feeding removes moisture from bulliform cells and consequently, leaves cannot expand and uncurl. WCM desiccate rapidly if exposed to high temperatures and low levels of humidity (Slykhuis 1955). Hence, mites inhabit only protected areas of the plant (Nault and Styer 1969). As

new leaves emerge, WCM move from the older to the newer leaves seeking protection.

During the spring, mites move to the flag leaf and subsequently to seed heads (Nault and Styer 1969).

When exposed to light WCM move to the inner whorl of wheat, so it was suggested that they are negatively phototrophic (del Rosario and Sill 1958). However, Nault and Styer (1969) observed that WCM exhibit a dispersal behavior as plants mature. This behavior, also described by Gibson and Painter (1957), consists of mites moving upward on the leaf and forming clusters of individuals. The mites assume a vertical position attaching their caudal sucker to the leaf and from there can be dispersed in clusters via air currents (Gibson and Painter 1957, Nault and Styer 1969). Hence, WCM can be either negatively or positively affected by light, depending on their physiological state (Nault and Styer 1969).

A critical factor for disease incidence is the dispersal of WCM to alternative hosts in the summer and into the new wheat in the fall. Virus infection is strongly correlated to the distance from original infection sources (Slykhuis 1955, Nault and Styer 1969). WCM depend on wind direction and speed for long distance dispersal (Slykhuis 1955, Staples and Allington 1956, Stilwell 2009). Also, WCM dispersal is strongly correlated with high mite populations, although some dispersal can still occur with small populations (Thomas and Hein 2003). As mites move from alternative hosts, there are two peaks of dispersal. The first one is in July and the other one is in late August, September or early October (Nault and Styer 1969). Dispersal rates to winter wheat slowly decline until November (Staples and Allington 1956). During the summer, mites disperse as wheat is approaching maturity (Liu *et al.* 2005).

### WCM Alternative Hosts

The primary host of the wheat curl mite is wheat, but mites can survive on a variety of other crops and grass hosts. Wild grass hosts for the mite include jointed goatgrass (*Aegilops cylindrica* Host.), western wheatgrass (*Agropyron smithii* Rydb.), wild oats (*Avena fatua* L.), grama (*Bouteloua* sp.), downy brome (*Bromus tectorum* L.), sandbur (*Cenchrus pauciflorus* Benth.), smooth crabgrass (*Digitaria ischaemum* (Schereb.)), barnyard grass (*Echinochloa crusgalli* (L.) Beauv.), Canada wild-rye (*Elymus canadensis* L.), stink grass (*Eragrotis cilianensis* (All.) Lutali), witchgrass (*Panicum capillare*), bristly foxtail (*Setaria verticillata* (L.) Beauv.), yellow foxtail (*Setaria glauca* L.), green foxtail (*Setaria viridis* (L.) Beauv.) and johnson grass (*Sorghum halepense* L.) (Connin 1956b, Slykhuis 1956, and Wegulo *et al.* 2007).

Corn, barley, sorghum, oats, rye, foxtail and pearl millets are considered important hosts for the WCM (Connin 1956, Wegulo *et al.* 2007). Corn can be a significant over-summering host (Nault and Styer 1969). WCM can survive well on leaves, husks, silk and kernels, with its peak abundance occurring in late July or early August (Nault and Styer 1969). The presence of the mites in corn is linked to corn discoloration known as Kernel Red Streak (Slykhuis 1968, Nault and Styer 1969, Liu *et al.* 2005). Some of these secondary hosts also serve as virus reservoirs. Therefore, alternative hosts perpetuate the disease cycle in the absence of wheat.

### Viruses Transmitted by the WCM

The WCM transmits WSMV, TriMV and WMoV to wheat in the Great Plains. The symptomology of these viruses is similar, making it hard to distinguish them in the field. Enzyme-linked immunosorbent assay (ELISA) and Polymerase chain reaction

(PCR) are used in the lab for identification. TriMV and WSMV in single or double infections have been shown to synergistically reduce yield and yield determinants of wheat (Byamukama *et al.* 2011, Byamukama *et al.* 2014). Surveys have demonstrated that double or triple infections in the field are common throughout the Midwest (Burrows *et al.* 2009, Byamukama *et al.* 2012).

### **Wheat streak mosaic virus**

WSMV was first recognized in Nebraska as yellow mosaic in 1922, but it caused its first extensive loss in the state in 1949 (Staples and Allington 1956). Impact of WSMV on winter wheat yield increases in years of warm falls and springs (Wegulo *et al.* 2007). WSMV symptoms on winter wheat usually do not appear until the spring as the plant becomes discolored, rosetted and stunted (Wegulo *et al.* 2007). Plants infected early in the season will show slight mottle symptoms, eventually turning into a mosaic pattern with uneven streaks along the leaf (Staples and Allington 1956). If infection occurs only in the spring, after plants are well tillered, WSMV symptoms will be faint (Wegulo *et al.* 2007). WSMV reduces wheat root biomass, thus decreasing water intake by the plant, which is a problem in areas with scarce water (Price *et al.* 2010).

Commonly grown crops and some wild grasses can also be infected by WSMV (Connin 1956b, Slykhuis 1956, Wegulo *et al.* 2007). While the impact of the virus on these alternative hosts is uncertain, it is commonly accepted that alternative hosts serve as virus reservoirs and as potential sources of genetic variation for mites and viruses.

The virus is transmitted via vector or seed. The WCM is the only known vector of WSMV (Connin and Staples 1957). Additionally, seed transmission has been shown to occur at 0.5-1.5%, depending on the wheat genotype (Jones *et al.* 2005). Seed

transmission is responsible for dispersal of WSMV across wheat growing regions of the world (Dwyer *et al.* 2007).

WSMV is a single stranded RNA with 9,384 nucleotides, excluding the polyadenylated tail (Stenger *et al.* 1998). WSMV previously belonged to the genus *Rymovirus*, but in 1998 it became the type species to the new genus *Tritimovirus* in the family *Potyviridae* (Stenger *et al.* 1998). WSMV is now present in nearly every world region where wheat is grown (Navia *et al.* 2012). WSMV was detected in 2002 in Australia (Ellis *et al.* 2003) and in Argentina (Troul *et al.* 2004). Phylogenetic analyses of different strains of WSMV in Australia indicate that the virus entered the country once via seed and multiplied from there (Dwyer *et al.* 2007). Using coat protein (CP) nucleotide sequence, it was established that WSMV from Australia, Argentina and North America belong to the same phylogenetic lineage (Stenger and French 2009).

While there are many strains of WSMV circulating in the world, many mutations are lost when wheat is harvested, hence the virus population undergoes genetic drift via this bottleneck effect (French and Stenger 2003). Choi *et al.* (2001) used the WSMV strains Type, Sidney 81 and El Batan 3 to study the evolution of the virus. Type and Sidney 81 have 97.6% shared nucleotide sequence and El Batan 3 shared ~79.3% nucleotide sequence with the other two strains (Choi *et al.* 2001). The variations among WSMV isolates are driven by negative selection and are considered neutral in respect to fitness (Choi *et al.* 2001, French and Stenger 2003).

### **Wheat mosaic virus**

Wheat mosaic virus, formerly known as High plains virus (HPV), was first identified in 1993/1994 in corn varieties in Texas, Nebraska, Kansas, Colorado, Idaho

and Utah (Jensen *et al.* 1996). Hosts for the virus include barley, cheat grass (*Bromus secalinus* L.), corn, oat, rye, yellow foxtail and green foxtail (Seifers *et al.* 1998). It belongs to the genus *Emaravirus*, family *Bunyaviridae* (Mielke-Ehret and Mühlbach 2012). Members of this genus have multipartite genomes of negative sense single stranded RNA (Mielke-Ehret and Mühlbach 2012).

WMoV is transmitted by the WCM and it is not mechanically transmissible (Seifers *et al.* 1997). WMoV transmission rates depend on the genotype of the mite. WCM collected from NE (Type 2) transmitted various strains of WMoV at high rates; however, mites isolated from MT (Type 1) transmitted WMoV, but at lower rates (Seifers *et al.* 2002). WCM isolated from Kansas (Type 1) poorly transmitted one strain of WMoV (Seifers *et al.* 2002). WMoV transmission by MT mites significantly increased when mites were also viruliferous for WSMV (Seifers *et al.* 2002). Seed transmission occurs in sweet corn at very low rate (Forster *et al.* 2001). However, seed transmission of WMoV on wheat and other hosts is not known.

Symptoms of WMoV on wheat are similar to those of WSMV starting with light chlorotic spots, turning into a mosaic pattern as the infection spreads (Jensen *et al.* 1996). Diagnosis of infected plants can be done via PCR or ELISA. It can also be diagnosed by a characteristic 32-kDa nucleoprotein encoded by RNA-s, which accumulates in the plant a few days post infection (Skare *et al.* 2006).

### **Triticum mosaic virus**

TriMV was isolated from wheat on the Kansas State University Agricultural Research Center in Hays, KS (Seifers *et al.* 2008). The virus belongs to the family *Potyviridae* and has 10,266 nucleotides, excluding the 3' polyadenylated tail (Tatineni *et*



*al.* 2009). Phylogenetic analysis based on amino acid sequences of polyprotein, Nib protein, NIa-VPg protein and coat protein placed TriMV as a sister clade of *Sugar cane streak mosaic virus* (SCSMV) (Tatineni *et al.* 2009). TriMV also has an extra long 5'-leader sequence, which is unusual for *Potyviridae*. Therefore, TriMV was placed under a new genus *Poacevirus* as the type member and SCSMV also as a distinct member (Tatineni *et al.* 2009). Fourteen TriMV isolates collected from Kansas, Oklahoma and Texas have nearly identical coat protein sequences (Fuentes-Bueno *et al.* 2011). Viruses that are well adapted to their host show higher levels of genetic variation (Fuentes-Bueno *et al.* 2011). Therefore, the authors hypothesize that TriMV was either recently introduced to wheat and is adapting, or that TriMV suffered a bottleneck effect and consequently has low genetic variability.

TriMV greatly reduces the yield and seed weight of susceptible of wheat cultivars such as 'Jagalene', 'Danby' and RonL (Seifers *et al.* 2011). Barley, oat, rye and triticale cultivars are also susceptible to TriMV (Seifers *et al.* 2010). Other hosts for TriMV include jointed goatgrass, wild oats, rye brome, cheat grass, field brome, prairie cupgrass and green bristle grass (Seifers *et al.* 2010). The impact of TriMV on these alternative hosts is not known.

The WCM was confirmed as the vector of TriMV with low transmission rates of 2.4%, but the mite genotype used was unknown (Seifers *et al.* 2009). Using single mites transfers, TriMV was successfully transmitted by Type 2 mites at a 41% rate (McMechan 2012). Type 1 mites only transmitted TriMV at a 2.5% rate only under very high mite numbers (McMechan 2012). Based on these results, Seifers and colleagues probably used Type 1 or a mixed source of mites for their study.

In field surveys, WSMV is more prevalent in single infections than TriMV or WMoV (Burrows *et al.* 2009, Byamukama *et al.* 2013). However, double and triple infections are common, and TriMV is most often found with WSMV (Burrows *et al.* 2009, Byamukama *et al.* 2013). Simultaneous co-infection of wheat by WSMV and TriMV can result in titer increase for both viruses and increased disease symptoms on specific wheat cultivars (Tatineni *et al.* 2010). The same authors also reported that WSMV concentration decreased at 28dpi and TriMV increased at 28dpi, in comparison to 14dpi for both single and double infections. This suggests that WSMV is aggressive in the initial phase of the infection and TriMV is constant, accumulating over time (Tatineni *et al.* 2010).

### **Vector Modes of Transmission**

Modes of transmission by vectors have been classified using a Hemiptera-virus relationship and are classified into non-persistent, semi-persistent, persistent circulative and persistent-propagative (Ng and Falk 2006). The classification is based on acquisition, latent and retention periods, transstadial and transovarial passage, virus circulation in the hemolymph, and virus replication in the vector (Ng and Falk 2006). Latent or incubation period is the time that it takes from acquisition to the ability of being infective. A non-persistent virus is stylet-borne and is acquired within seconds to minutes and retained for only minutes or hours (Ng and Falk 2006). Semi-persistent viruses are acquired within minutes to hours and can be retained in the vector for hours to days, but the virus is not carried through molting nor it is passed to its offspring. Persistent-circulative viruses are acquired and have latent periods of hours, and the viruses remain in the vector from days to throughout the vector's life span. The virus circulates in the hemolymph, so it is

retained through vector molting (Ng and Falk 2006). The last category, persistent-propagative viruses, has the same acquisition, retention, circulation and transstadial passage characteristic as persistent-circulative. The difference between the two is that persistent propagative viruses have a latent period that can last from days to weeks, the virus replicates within the vector and in some cases can be passed to offspring (Ng and Falk 2006).

Eriophyid vectors have a close relation to the transmitted virus, most exclusively transmitting only one virus (Oldfield and Proeseler 1996). The only known eriophyid mites that transmit more than one virus are the WCM and *Abacarus hystrix* Nalepa, the cereal rust mite (Oldfield and Proeseler 1996). Wheat streak mosaic virus is transmitted transstadially, but not transovarially (Siriwetwivat 2006). About 1% of mites can acquire the virus within 15 minutes of feeding, but virus acquisition efficiency increases with feeding time (Orlob 1966). Nymphs and larvae can acquire the virus, but adults cannot acquire the virus (Orlob 1966). Rates of transmission of WSMV on plants with surviving mites are higher for nymphs (82.8%) and molting mites (94.4%) than in adults (50%) (Siriwetwivat 2006). WCM remained infective for 7 days at 23 -28°C and for 61 days at 3°C, but the authors noted that infectivity decreases with mite's age (Orlob 1966).

Paliwal (1980) indicated that WSMV has a semi-persistent mode of transmission, since it can persist intact in the mite's midgut for up to 5 days. WSMV was also found in the haemocoel and in the salivary glands of the WCM suggesting that mites could inject the virus along with its saliva (Paliwal 1980). The author then suggested that the virus could be circulative, but he did not discard the possibility of transmission by regurgitation. Using the Ng and Falk (2006) classification, WSMV would be classified as

semi-persistent, since there is no latent period of WSMV transmission. But WSMV is retained through molts. Thus, using the same classification it would be classified as persistent-circulative. One issue is that mode of transmission of plant viruses is based on vector-virus relationships with hemipteran vectors. Mode of transmission for the WCM-WSMV system is not clear and more studies should be performed to solve this uncertainty.

It is known that the WCM needs the helper component-proteinase (HC-Pro) of WSMV for transmission (Stenger *et al.* 2005). HC-Pro is a multi-functional protein present in *Potyviridae* and has many functions, including aphid transmission of viruses and virus movement in the plant. TriMV and WMoV modes of transmission have not been studied. But, TriMV is thought to be retained transstadially and is not transovarially transmitted (McMechan 2012).

### **Virus-Host-Vector Relations**

Arthropod-borne plant viruses exhibit a close relationship with their vector, and vector performance is often improved on infected host plants. For example, the Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), feeding on *Tomato spotted wilt virus* (TSWV) infected pepper plants had higher juvenile survival and faster developmental rates than non-infected thrips (Belliure *et al.* 2005). Higher juvenile survival not only leads to more reproductive adults, but it also increased the chance of virus dispersal (Belliure *et al.* 2005).

Also, pathogen-induced symptoms such as yellow or mottled leaves and enhanced volatile emissions can increase vector attraction to infected plants (Bosque-Perez and Eigenbrode 2011). Eigenbrode *et al.* (2002) found that the green peach aphid (GPA),

*Myzus persicae* Sulzer, settled and aggregated preferentially on plants infected with *Potato leaf roll virus* (PLRV), but did not show this behavior on plants infected with *Potato virus X* and *Potato virus Y* (PVX and PVY, respectively) to which GPA is not a vector. Because the study was conducted in the dark and prevented the aphids from directly contacting the plants, the findings suggest that vector specific volatiles emitted by virus infected plants attract more vectors than virus free plants and aid in their own dispersal.

Mode of transmission may also be a determinant in pathogen-plant-vector interactions. Non-persistent viruses are stylet-borne and only remain in the vector for minutes to hours (Ng and Falk 2006). Hence, viruses transmitted in this manner may benefit by attracting vectors but providing them with sub-optimal plant resources. The vector will probe and acquire the virus, but it will disperse rapidly, looking for a better host and consequently spreading the virus (Mauck *et al.* 2012). For example, *Cucumber mosaic virus* (CMV) decreases the quality of cucumbers, but infected plants are more attractive to CMV aphid vectors, *M. persicae* and *Aphis gossypii* Glover (Mauck *et al.* 2010). Increased attractiveness of aphids to infected plants was driven by elevated volatile emissions that were similar to those emitted by healthy plants. Thus, the aphids would land on an infected plant, and upon detection of lower quality, would rapidly disperse and aid in virus dissemination (Mauck *et al.* 2010).

Persistently-transmitted pathogens, may benefit vectors by improving host plant quality since extended feeding on infected plants is required for vectors to successfully acquire a pathogen and become infectious (Mauck 2012). Such is the case with increased fitness of WFT after feeding on TSWV infected pepper plants (Belliure *et al.* 2005). This

holds true for potato plants infected with PLRV transmitted by *M. persicae*. Infected plants were superior hosts and triggered higher growth and reproductive rates for GPA when compared to aphids reared on virus free plants (Eigenbrode *et al.* 2002). WSMV also follows this pattern, significantly increasing the reproduction rate of the WCM (Siriwetwivat 2006). Mite populations feeding on WSMV infected plants were found to build up to three times faster than populations feeding on virus-free plants (Siriwetwivat 2006).

Decreases or neutral effects in arthropod fitness also occur in pathogen-vector systems. TriMV decreased the reproduction rate of WCM on infected wheat (McMechan 2012). Pea aphids, *Acyrtosiphon pisum* (Harris), reared on *Pea enation mosaic virus* infected tic beans (*Vicia faba* L.) showed no changes in reproduction survival or growth (Hodge and Powell 2008). However, when tic beans were infected with *Bean yellow mosaic virus* (BYMV) aphid survival was decreased (Hodge and Powell 2008).

### **Virus-Virus Interactions**

Many studies of plant viruses are focused on individual interactions between the virus and the host plant or the vector. However, multiple viral infections in plants are common in nature and in agricultural fields. Varying degrees of synergism or antagonism can occur as a result from mixed infections. Intra-host interactions among viruses can be neutral, beneficial or disadvantageous with respect to virus fitness. The interactions can depend on host species, cultivar, temperature or time of infection of each virus.

#### **Synergism**

Synergism among co-infecting viruses occurs when there is an increase in replication or increased virulence of one or both viruses (Roossinck 2005, Syller 2012). It

is thought that most synergistic relations occur between unrelated viruses (Roossinck 2005, Syller 2012). One virus can exhibit synergistic relationships with several unrelated viruses. For example, *Sweet potato chlorotic stunt crinivirus* (SPCSV) can be synergistic with several viruses including; Ipomovirus (*Sweet potato mild mottle virus* ‘SPMMV’), Cucumovirus (CMV) and Carlavirus (C-6 virus) (Untiveros *et al.* 2007). Each virus by itself shows mild or no symptoms and is hard to detect serologically. But when co-infected with SPCSV, symptoms become apparent and replication and movement in the plant increase (Untiveros *et al.* 2007). Plant symptoms are often worsened when synergetic co-infection is present. Moreover, because many viruses show increase in titers, many viruses that go unnoticed in single infections are detected in dual infections.

Several studies involve members of Potyviridae, the biggest and most economically important family of plant viruses and heterologous virus family (Roossinck 2005, Syller 2012). There is a general consensus that in mixed infections potyvirus titer remains unaffected while the other virus increases in concentration, although exceptions can occur (Pruss *et al.* 1997, Syller 2012). When inoculated simultaneously, PVX (Alphaflexiviridae) had up to ten times the concentration of PVY (Potyviridae); however, PVY concentration remained the same on single or double infected plants (Rochow and Ross 1955). The same pattern is seen with *Soybean mosaic potyvirus* (SMV), increasing the titer of two Comoviridae viruses; *Cowpea mosaic virus* and *Bean pod mottle virus* (Anjos *et al.* 1992).

Certain synergistic relations can be beneficial to both viruses. Co-infection of corn with *Maize chlorotic mottle virus* (MCMV) (Tombusviridae) and WSMV causes corn lethal necrosis disease (CLND). Both WSMV and MCMV show an increase in titer

when they co-infect corn (Stenger *et al.* 2007). WSMV shows up to six-fold increase in titer 17 days post inoculation (dpi), while MCMV increased up to four-fold in titer when compared to a singly inoculated treatment, but this only occurred at 28 dpi (Stenger *et al.* 2007).

Mixed infections of two potyviruses are uncommon, but when they occur they result in synergism. In comparison with single inoculations, co-infection with WSMV and TriMV resulted in up to a 7.4-fold increase in titer in susceptible wheat cultivars (Tatineni *et al.* 2010). WSMV is also found to be synergistic with the potyvirus *Agropyron mosaic virus* (AMV) (Slykhuis and Bell 1966). WSMV infected with AMV, simultaneously or 10 days apart, increased symptom severity, indicating synergism among the species (Slykhuis and Bell 1966).

Disease synergism is dependent on several factors, including abiotic factors and host and virus characteristics. Dual-infection synergism can be temperature and cultivar dependent. In the susceptible cultivars Tomahawk and Arapahoe, WSMV and TriMV co-infection greatly increased each virus concentration and exacerbated symptoms (Tatineni *et al.* 2010). Using the WSMV resistant cultivar Mace, synergism was temperature dependent. At 19°C Mace conferred resistance against WSMV and surprisingly to TriMV as well. But when kept at 20-26°C both WSMV and TriMV were able to replicate and showed mild symptoms (Tatineni *et al.* 2010). Increases in viral titer can be host-dependent. PVX had significantly increased titer when co-infection occurred with potyviruses (PVY and TEV- *Tobacco etch virus*) inoculated on *Nicotiana tabacum* L., but titer did not increase over single infections on *Nicotiana benthamiana* Domin (Gonzalez-Jara *et al.* 2004). Symptoms were more aggravated in *N. benthamiana* than in



*N. tabacum*, suggesting that titer of PVX is not the primary factor for symptom development (Gonzalez-Jara *et al.* 2004).

Pathways of synergism in Potyviruses are usually mediated by the expression of helper component-proteinase (HC-Pro) (Syller 2012). HC-Pro aids in vector transmission and also functions as a silencing suppressor of host's post transcriptional gene silencing (PTGS) (Stenger *et al.* 2007, Syller 2012). PTGS uses small interfering RNA (siRNA) to degrade virus RNA. The Potyvirus, *Turnip mosaic virus* (TuMV) when it co-infects simultaneously with the Crinivirus *Lettuce infectious yellows virus* (LIYV) produces symptoms in *Nicotiana benthamiana* plants resulting in increased LIYV replication efficiency and titer (Wang *et al.* 2009). Transgenic plants of P1/HC-Pro, an RNA-silencing suppressor, inoculated with LIYV showed similar symptoms to the LIVY+TuMV infected plants, suggesting that TuMV has silencing suppressor roles and mediates the increase in LIVY titers (Wang *et al.* 2009). Different genera in the family Potyviridae have limited HC-Pro sequence and not all members use HC-Pro equally (Stenger *et al.* 2007). WSMV lacking HC-Pro still engaged in synergism with MCMV (Stenger *et al.* 2007). In this case, other genes are thought to be responsible for PTGS silencing. Other viral proteins are thought to suppress host RNA-silencing and ultimately dictate synergistic patterns among co-infecting viruses (Syller *et al.* 2012).

Mixed infections are necessary for specific viruses to be transmissible. Helper dependence occurs when a dependent virus is only transmitted in the presence of a second helper virus (Rochow 1972). This phenomenon is common in the genus *Umbravirus*. These species lack capsid protein that is required for aphid transmission (Syller 2012). Umbraviruses are transmissible if they co-infect with a member of

*Luteovirus*, because the helper virus encapsidates the umbraviral RNA, making it aphid transmissible (Syller 2012). In other systems, HC-Pro can assist in aphid transmission of a virus that is usually non-transmissible (Syller 2012). An active Potyvirus HC-Pro can bind to a non-transmissible virus and mediates its transmission (Syller 2012).

### **Antagonism**

Virus-virus antagonism happens when one virus benefits at the expense of the other, lowering the fitness of the second virus (Syller 2012). *Brassica* spp. plants co-infected with TuMV and *Cauliflower mosaic virus* (CaMV) showed a 77% increase of TuMV and a 56% decrease of CaMV in comparison to their single infected counterparts (Martin and Elena 2009). The mechanism for this particular process is not clear. But the authors suggest that either TuMV is using shared resources more efficiently than CaMV and/or TuMV elicits host responses that harm CaMV. It is interesting to point out that this kind of negative interference in the long term can reduce titers and potentially drive a virus species or strains to extinction .

Cross-protection or ‘super-infection interference’ works like a ‘vaccine’ to the plant. A mild virus infects the plant, and that plant becomes resistant to the symptoms produced by the latter virus inoculation (Syller 2012). The first virus is called the protecting virus and the second is called the challenging virus. The more similar the virus species or strains, the greater likelihood that cross-protection will occur (Rossinck 2005). Using *Beet soilborne mosaic virus* (BSBMV) and *Beet necrotic yellow vein virus* (BNYVV), both from the *Benyvirus* genus, BSBMV was able to confer cross-protection against BNYVV at 5 and 10 day inoculation intervals, and at 10 day intervals they found 100% cross-protection (Mahmood and Rush 1999). Slykhuis and Bell (1966) showed

cross-protection among three different isolates of WSMV. If wheat was first inoculated with the mild isolate WSMV-Kmi and infected 10 days later with WSMV-A or WSMV-Ks then these plants developed similar symptoms to single inoculated plants, instead of increasing in symptom severity (Slykhuis and Bell 1966).

Antagonistic strains or species can avoid co-existence in the same cell by mutual exclusion (Syller 2012). This is referred to as spatial separation. Methods for detecting spatial separation involve fluorescent labeling and analysis of cell maps to determine interaction among viruses. Using this method, Dietrich and Maiss (2003) determined the existence of spatial segregation among *Tobacco vein mottling virus* (TVMV) and *Plum pox virus* and between TVMV and *Clover yellow vein virus*. In this study, most replication of the different viruses occurred in discrete areas of neighboring epidermal cells, although some co-existed in the same cell clusters. Similar fate can be found with identical strains of apple latent spherical virus (ALSV) expressing either yellow or cyan fluorescent proteins (ALSV-YFP and ALSV-CFP, respectively). ALSV-YFP was inoculated at 3, 5, 6, and 11 days post-inoculation of ALSV-CFP, and they found the labeled virus populations were always segregated in leaves. Thus, ALSV-YFP could only infect where ALSV-CFP was not established (Takahashi et al. 2007). Elena (2011) suggests that mutual exclusion can reduce recombination rates, thus limiting sources of genetic variation. However, the concept of spatial segregation is still relatively new and the mechanisms behind it are still unknown.

### **Viral Interactions Impact on Epidemiology**

Few studies have been performed on the impact of mixed infections on virus transmission and vector biology. Virus concentration in the host is a major factor in

arthropod acquisition, hence if a virus increases in concentration in mixed infections there is a greater likelihood it will be transmitted. Mixed infections of *Tomato chlorosis virus* and *Tomato infectious chlorosis virus* resulted in increased titer of both viruses in *N. benthamiana* and lower titers of both viruses in *Physalis wrightii* (A.) Gray (Wintermantel *et al.* 2008). The whitefly vector *Trialeurodes abutilonea* (Haldeman) had increased transmission efficiency of each virus when titers increased, but transmission rates were decreased when titers decreased (Wintermantel *et al.* 2008).

One virus can also benefit if another virus induces host changes that attract the vector. Potato plants infected with both PVY and PLRV attracted more alates and apterae vectors (*M. persicae* and *Macrosiphum euphorbiae* (Thomas)) than single or non-infected plants (Srinivisan and Alvarez 2007). Mixed infection also increased fecundity of both vectors when compared to non-infected and single PVY treatments (Srinivisan and Alvarez 2007). *Watermelon mosaic virus* (WMV) decreases in concentration if it co-infects squash *Cucurbita pepo* L. cv. ‘Dixie’ with *Zucchini yellow mosaic virus* (ZYMV) (Salvaudon *et al.* 2013). *A. gossypii*, however, did not alter transmission rate of WMV in mixed infection treatments. ZYMV enhanced plant volatile emission and yellowing of leaves, and both attracted more *A. gossypii* than WMV infected plants and controls (Salvaudon *et al.* 2013). The authors argue that the reduction of WMV titer in the presence of ZYMV is compensated for by the enhanced aphid traffic dual infected plants receive, thus spreading the disease further.

Symbiotic relations among arthropod-borne viruses can induce changes in fecundity, transmission and dispersal of the vector. Any change in the vector directly impacts epidemiology and require more attention in management strategies. Synergistic

relations can transform an unimportant virus into an important disease complex.

Disease complexes such as sweet potato virus disease (SPVD) and corn lethal necrosis disease (CLND) are caused by synergism among viruses and greatly impact yield (Untiveros et al. 2007, Stenger *et al.* 2007). Synergism among viruses can also cause viruses to overcome plant resistance. The variety of East African sweet potato, cv. ‘Tanzania’, is resistant to *Sweet potato feathery mottle potyvirus* (SPFMV) and mildly susceptible to *Sweet potato chlorotic stunt crinivirus* (SPCSV). When these two viruses occurred together, SPFMV was able to systemically infect sweet potato, thus the synergism resulted in resistance breakdown in Tanzania (Karyeija *et al.* 2000). Interestingly, the potyvirus in this case is the beneficiary and the crinivirus is the helper; however, potyviruses are usually unaffected by synergistic relations (Syller 2012). Movement of SPFMV didn’t change in the presence of SPCSV, thus the increase in SPFMV was possibly due to SPCSV breaking down host resistance and enhancing SPFMV multiplication (Karyeija *et al.* 2000).

*Tomato chlorosis virus* (ToCV), a crinivirus, and *Tomato spotted wilt virus* (TSWV), a tospovirus, co-infection in tomatoes can overcome resistance (Garcia-Cano et al. 2006). They found TSWV resistance in tomato cv. ‘Anastasia’ broke down when plants were inoculated with ToCV and super-infected with TSWV 10 days later. Resistance in this case is due to the gene Sw-5 that provides broad-spectrum resistance to tospoviruses. It is speculated that ToCV represses the resistance conferred by Sw-5 and ultimately enhances plant susceptibility to TSWV (Garcia-Cano et al 2006).

Alternatively, the presence of one virus can negatively impact the success of the other. The luteovirus barley yellow dwarf virus (BYDV), has two strains, MAV and

PAV, that historically alternate in prevalence in field samples. When they co-infect in oats, PAV interferes with MAV's replication, resulting in lower MAV concentration (Power 1996). In single infections PAV has a higher transmission rate than MAV by the aphid species *Rhopalosiphum padi* (Power 1996). Given that high BYDV concentrations are essential for virus transmission by the aphid vectors (Gray et al. 1991), low concentration of MAV directly impacts its spread in the field. On the other hand, oats susceptible to BYDV inoculated with MAV or PAV at least fifteen days before the other strain, become resistant to the other strain (Power 1996).

Virus-virus interactions greatly impact epidemiology and in turn disease management. In the WCM-virus disease complex, these interactions can enhance transmission of a virus and break host plant resistance. MT (Type 1) WCM increased the rate of WMoV transmission if they were viruliferous for WSMV (Seifers *et al.* 2002). Dual infection of WSMV and TriMV worsens symptoms and increases replication of both viruses on select cultivars (Tatineni *et al.* 2010). How mixed infections affect WSMV and TriMV transmission rates and vector biology is the subject of this thesis.

**Chapter 2: Impact of *Wheat streak mosaic virus* and *Triticum mosaic virus* co-infection of wheat on transmission rates by Type 1 and Type 2 wheat curl mites**

## Introduction

The wheat curl mite (WCM), *Aceria tosichella* Keifer, is the only known vector of *Wheat streak mosaic* (WSMV), *Wheat mosaic* (WMoV) and *Triticum mosaic* (TriMV) viruses (Slykhuis 1955, Seifers *et al.* 1997, Seifers *et al.* 2009). WCM populations are made up of different strains, biotypes and genotypes. Host strains of the WCM were shown when mites reared on *Hordeum jubatum* L., *Elymus canadensis* L. and *Agropyron smithii* Rydb. could not survive on wheat and vice-versa (Slykhuis 1955). Virus transmission differences were demonstrated when mites reared on *A. smithii* transmitted WSMV at significantly lower rates than mites from wheat (del Rosario and Sill 1965). Once these mites adapted to wheat they transmitted WSMV at comparable rates to those colonies that were always reared in wheat (del Rosario and Sill 1965).

Biotypic differences in WCM response to wheat resistant lines have been observed (Harvey *et al.* 1995b, Harvey *et al.* 1997 and Harvey *et al.* 1999). WCM genotypes have been classified into Type 1 and Type 2 based on ribosomal and mitochondrial DNA (Carew *et al.* 2009, Hein *et al.* 2012). A few isolated mites have been found to have Type 1 and Type 2 characteristics, indicating that mite genotypes might be able to interbreed (Carew *et al.* 2009). Mite colonies originally isolated from collections made in South Dakota (SD), Montana (MT), Texas (TX), and Kansas (KS) were shown to be Type 1 WCM, while a colony originating from a Nebraska (NE) collection was shown to be Type 2 WCM (Hein *et al.* 2012). WCM genetic differences correlate to some of the findings of different biotypes and differential virus transmission (Hein *et al.* 2012).



These WCM genotypes have been shown to transmit viruses differentially. In the U.S., both mite genotypes were shown to transmit WSMV at varying rates (Seifers *et al.* 2002). However, in Australia, only Type 2 mites were able to transmit WSMV (Schiffer *et al.* 2009). Type 2 mites (NE) transmitted WSMV at an average rate of 43 to 68%, depending on the vector's phenological stage (Siriwetwivat 2006). WMoV was transmitted by Type 1 mites (KS, TX, SD and MT) at lower rates than Type 2 (NE) mites (Seifers *et al.* 2002). TriMV was transmitted by single Type 2 mites at a rate of 41%, but could only be transmitted by Type 1 mites at a low rate (2%) using very high populations (McMechan 2012).

WSMV (genus *Tritimovirus*, family *Potyviridae*) was first identified in Nebraska in 1922, but caused its first extensive loss in winter wheat in 1949 (Staples and Allington 1949) and it has caused sporadic but extensive losses ever since. WMoV (genus *Emaravirus*, family *Bunyaviridae*) was isolated in 1993/1994 in corn, but it is also virulent to wheat (Jensen *et al.* 1996). Lastly, TriMV (genus *Poacevirus*, family *Potyviridae*) (Tatineni *et al.* 2009) was first identified in 2006 in WSMV resistant wheat lines (Seifers *et al.* 2008). These three viruses produce similar symptomology on wheat (*Triticum aestivum*), causing mosaic discoloration, rosetting and stunting of the plant.

Kansas' disease report estimated that the average loss due to the WCM disease complex (WSMV, TriMV and WMoV) was 1% through the past 20 years (Appel *et al.* 2013). But heavily affected fields can have 100% yield loss. In field surveys, WSMV is the most prevalent of these viruses, followed by WMoV and TriMV (Burrows *et al.* 2009, Byamukama *et al.* 2013). Triple infection by the viruses can occur at low rates, but double infections are more common (Burrows *et al.* 2009, Byamukama *et al.* 2013).

While WSMV is most commonly found in single infections in the field, TriMV infections appear to be dependent on WSMV as 91% of TriMV positive samples were co-infected with WSMV (Byamukama *et al.* 2013). WSMV and TriMV exhibit synergism when they co-infect, with greater symptom expression and increased titers of both viruses (Tatineni *et al.* 2010).

Co-infection with WCM transmitted viruses can impact transmission rates of individual viruses. Type 1 mites (MT only) reared on barley transmitted different strains of WMoV at low rates, but transmission efficiency increased when using WSMV viruliferous mites (Seifers *et al.* 2002). Using an unknown mite source, Seifers *et al.* (2009) observed that TriMV transmission rate increased when the source plant was co-infected with WSMV.

Given the increases in transmission rates of WMoV and TriMV in the presence of WSMV, a better understanding of the nature of these viral co-infections and their impact on transmission and epidemiology is needed. There is also a need to evaluate WSMV transmission in the presence of another virus. The objective of this study was to investigate how double viral infections with WSMV and TriMV impact transmission rates of each virus for both Type 1 and Type 2 WCM.

## Materials and Methods

Established colonies, MT and SD (Type 1) and NE (Type 2), were used for these studies. Mite colonies were maintained under artificial lights (14L:10D) either in a growth chamber or in a colony room maintained at ca. 22°C. Colonies were maintained on cv. 'Millennium' wheat grown in 15-cm dia pots. Mites were regularly transferred (ca. every 3 weeks) to new wheat plants. Cylindrical cages were placed over each pot in the colony to prevent contamination. These cages contained two vents on opposite sides and an open top all covered with Nytex® screen (250-micron mesh opening; BioQuip Products, Rancho Dominguez, CA).

**Co-transmission by Type 2 WCM.** Millennium wheat was seeded into 4-cm-dia cone-tainers™ (Stuewe & Sons Inc., Tangent, Oregon, USA) filled with autoclaved greenhouse soil. Plastic cylindrical cages (5-cm in diameter and 50-cm in height) with two to three Nytex® vents were used to cover the cone-tainers. Three source plants (replicates) for each of the four treatments were inoculated with sterilized water (mock), TriMV, WSMV or WSMV+TriMV at 21 days after seeding. A stock solution of 1:10 wt./vol. ratio of infected plant tissue/sterilized water was made for each virus. For single inoculations, 10 ml of the stock solution were combined with 10 ml of sterilized water. For double inoculations, 10 ml of each virus stock solution were combined. Thus, all inoculations resulted in a 1:20 plant/water ratio. Plant tissue for the inoculations was ground using a mortar and pestle. Plants to be inoculated were sprinkled with carborundum to allow scarring of the plant tissue and initiation of virus infection. Rub inoculation was performed by using the pestle, dipped in the inoculum and rubbing the leaf tissue against the palm of one hand.

Within a week after inoculation, 10 WCM were placed on a point-mount triangle (ca. 11 mm long vs. 3mm base) and carefully placed into the leaf axil of the newest leaf on each source plant. A mite transfer tool, made from a wood dowel with a single human eyelash attached, was used for mite transfers. Plants were then placed in a growth chamber (14L:10D) maintained at 27°C for two weeks.

After two weeks, single mite transfers from these source plants to 14-day old test plants were performed. To establish transmission rates, 10 single mite transfers were done for each source plant. For this process, source plants were cut and viewed under the microscope and mites were picked up with the transfer tool. A test plant was placed on an adjacent microscope and one mite was visually transferred to the whorl of the newest leaf. Only large (adult or late nymphal) mites exhibiting normal movement were used. Test plants were covered with cages and left overnight to allow mites to establish on the plant, then they were transferred to a growth chamber held at 27°C. After the single mite transfers, the source plants were sampled and stored at -20°C and tested for WSMV and TriMV via enzyme-linked immunoabsorbent assay (ELISA). Only test plants coming from sources positive for the respective viruses and negative for mock were analyzed.

Single mite transfer test plants were harvested 21-24 days after infestation. Mite presence or absence was determined for each of the test plants. At harvest, leaf pieces from test plants were sampled, and stored at -20°C until (ELISA) testing. Samples were tested for WSMV and TriMV with double antibody sandwich ELISA (DAS-ELISA). Because of the extensive labor involved in mite transfers, the number of replicates for each run was limited to three, but this was conducted four times for a total of 12 source plants for each treatment. Data were analyzed using direct comparisons of transmission

rates between WSMV vs. WSMV+TriMV and TriMV vs. WSMV+TriMV. PROC GLIMMIX (SAS) was used specifying a binomial distribution, Type 3 test of analysis of variance and least significant differences for virus presence. Mite survival vs. treatment interactions were also tested. Treatment effects and interactions at  $P \leq 0.05$  were considered significant.

**Co-transmission by Type 1 and Type 2 WCM.** For this study our objective was to determine virus transmission rates for Type 1 and Type 2 WCM feeding on double infected wheat plants. Conetainer planting, inoculation procedures and mite transfers were performed as described in the previous section. This experiment was conducted two separate times. The first run included three source plants each for a mock and WSMV+TriMV treatment for each of three WCM colonies tested, Type 2 (NE) and Type 1 (MT, SD). For the second run, the mock was eliminated to enable testing of six more source plants. This was also done because of the lack of contamination on the first run. In each run, viruliferous treatments had 10 individual mites transferred from the source plant to separate test plants, but in run one the mock only had 5 WCM transfers per source plant. The experiment included 90 test plants for WSMV+TriMV vs. NE and SD colony and 80 test plants for WSMV+TriMV vs. MT. The MT colony only had 80 test plants for the WSMV+TriMV combination because one source plant tested negative for WSMV.

Test plants were harvested 21 days after single mite transfers in the first run and only 14 days post WCM transfers in the second run, due to advanced symptom development. Mite survival on test plants was determined for each of the test plants. Leaf pieces of test plants were placed into mesh bags and stored at  $-20^{\circ}\text{C}$  until ELISA testing.

The samples were tested for WSMV and TriMV with double antibody sandwich ELISA (DAS-ELISA). Data were analyzed as described in the previous section.

**Virus assay.** Indirect ELISA for WSMV and TriMV was performed for all wheat test plants. For each sample, ca. 0.15-0.2 g of plant tissue was added to a mesh bag (Agdia, Elkhart, IN). General Extraction buffer (GEB) (Agdia) was added to mesh bags at a 1:10 wt./vol. ratio and then tissue was ground using a tissue homogenizer (Agdia). WSMV and TriMV tests of a sample set occurred simultaneously and tissue extracted from the same mesh bag was used for both tests. ELISA plates (96 well flat – Bottom Immuno Plate, Maxisorp, Nunc, Thermo Scientific Inc. Dubuque, IA) were coated with 100  $\mu$ l/well of primary antibody solution and stored overnight at 4°C. TriMV IGG (University of Nebraska-Lincoln; Dr. Satyanarayana Tatineni) at 1:1000 wt./vol. or WSMV capture antibody (Agdia) at 1:400 wt./vol. ratio were diluted into carbonate buffer 10X (Agdia). The following morning, plates were rinsed 3 times PBST 1X (Agdia). Extract (100  $\mu$ l) of each sample was added to each of two wells of the WSMV and TriMV plates and incubated for 1 hr at 37°C. Plates were rinsed (TriMV - 3 times; WSMV - 7 times) with PBST 1X (Agdia). Conjugate antibody diluted in GEB (100  $\mu$ l/well) was added to the plates at 1:400 wt./vol. ratio for WSMV and 1:500 wt./vol. ratio for TriMV IGG-ALP, and incubated for 1hr at 37°C. Plates were rinsed again (TriMV - 3 times; WSMV - 7 times) with PBST 1X (Agdia). PNP 5X buffer (100  $\mu$ l/well) (Agdia) was added, and plates were incubated at room temperature in the dark for at least one hour. Absorbance estimates at 405 nm were obtained with a Multiskan FC Spectrophotometer (Thermo Scientific Inc. Dubuque, IA). Absorbance values two times or higher than the negative control were considered positive.

## Results

**Co-transmission by Type 2 WCM.** All treatment source plants used in this study tested positive via ELISA for the respective viruses. All mock source plants and test plants tested negative for both WSMV and TriMV, indicating that no cross-contamination occurred between treatments. There was no significant treatment by run interaction, so data were combined across all four runs ( $F=0.49$ ,  $Pr>F=0.6868$ ). The virus assay for WSMV indicated that Type 2 WCM from single inoculation source plants transmitted the virus at a 50.0% (SEM 5.7) rate (Table 2.1). WCM feeding on plants co-infected with WSMV and TriMV transmitted WSMV at a mean percentage of 35.6% (SEM 5.3) (Table 2.1). WCM feeding on single inoculated TriMV had a transmission rate of 43.3% (SEM 5.6) (Table 2.1). Mites feeding on plants inoculated with WSMV and TriMV had total TriMV transmission rate of 56.8% (SEM 5.6) (Table 2.1). From the double inoculated source plants, 23% of the WCM transmitted both viruses, 33% transmitted TriMV alone and only 12.5% transmitted WSMV alone (Fig. 2.1). Transmission efficiency of WSMV suffered a significant reduction when TriMV was present in the source plant (50% vs. 35.6%,  $Pr>|t|=0.0274$ , Table 2.2). The opposite occurred for TriMV. Its rate of transmission by the WCM was significantly increased when WSMV was present in the source plant (43.3 % vs. 56.8%,  $Pr>|t|=0.0425$ , Table 2.2). Overall we have an odds ratio of 0.55 for WSMV transmission from double infected plants over single inoculated plants (Table 2.2.). This means that the chances of WSMV transmission occurring from mites feeding on double inoculation plants are 0.55X less in the co-infected treatment. TriMV transmission odds-ratio is 1.72 for double infected over TriMV alone plants, which

means that TriMV transmission is 1.7X more likely to be transmitted when the plant is co-infected with the two viruses (Table 2.2).

We then analyzed mite survival on the test plants. WCM survival rates on the test plants were highest for WSMV (65%), followed by mock (55%), TriMV (40%) and lastly double infected plants (37%). WSMV and mock survival rates were not statistically significant from one another, but they were significantly higher than TriMV and double infected treatments. We saw no significant mite survival by treatment interaction for TriMV transmission ( $F=0.64$ ,  $Pr>F=0.4235$ ). However, there was a significant mite survival by treatment interaction on WSMV transmission rates ( $F=4.33$ ,  $Pr>F=0.0386$ ). Hence, if we account for mite presence, WCM did not significantly differ in WSMV transmission rates between the WSMV and the WSMV+TriMV treatments. If mites survived, the rate of WSMV transmission was 59.7% when mites were feeding on WSMV-only infected plants and 68% when mites fed on co-infected plants ( $Pr>|t|>0.005$ ) and 31.8% and 16.13% ( $Pr>|t|>0.005$ ), respectively, if they did not survive (Table 2.3).

**Co-transmission by Type 1 and Type 2 WCM.** SD and MT colonies (Type 1) were unable to transmit TriMV, but NE (Type 2) mites transmitted TriMV at a 47.5% rate. We found no colony by run interaction ( $F=0.45$ ,  $Pr>F=0.64$ ) so WSMV transmission data were combined across runs. NE mites transmitted WSMV at a 45.5% rate, SD mites transmitted WSMV 36.5% of the time, and MT WCMs transmitted WSMV 20.9% of the time (Table 2.4). NE and SD transmission rates were not significantly different from each other, but they were both significantly different than MT transmission rate (Table 2.4). We found no colony by mite survival interaction ( $Pr>|t|=0.98$ ). Mite survival in the test



plants of the double inoculated treatments was 61% (SD), 54% (MT) and 42% (NE), only SD WCM survival was significantly different than NE survival ( $P_{>|t|} = 0.0102$ ). For the mock treatment (run 1) mite survival rates were 40% (SD), 33% (MT) and 66% (NE).

## Discussion

We observed that co-infection with WSMV and TriMV in the source plant can alter the transmission efficiency of each virus by Type 2 (NE) WCM when compared to single infected source plants. The WCM transmission rate of TriMV when WSMV was present in source plants was significantly reduced. WSMV, however, had significant reduction of transmission when TriMV was co-infected in source plants. Hence, TriMV transmission was benefitted and WSMV transmission was hindered when mites fed on double infected plants. Using Type 1 mites we did not see any TriMV transmission, supporting the findings of McMechan (2012).

Using single mite transfers, Seifers *et al.* (2009) indicated that WSMV could boost the transmission of TriMV from 2% in single inoculated plants to 18.6% in co-inoculated plants. Our data confirm these findings as the TriMV rate of transmission increased from a mean percentage of 43.3% in single inoculations to 56.6% in co-inoculated plants using Type 2 WCM. Mite genotype was not known for the Seifers *et al.* (2009) study, but it was most certainly not isolated Type 2 mites as the data are inconsistent with this study and to the study of McMechan (2012).

TriMV was strongly associated with WSMV in the Great Plains, being detected primarily in double infections (Byamukama *et al.* 2013). Our data show that TriMV increases in transmission efficiency when WSMV was present. However, this depends on the WCM genotype. We showed that only Type 2 (NE) mites were able to transmit both viruses at the same time. TriMV, as previously shown by McMechan (2012), was only transmitted by Type 2 mites, and WSMV does not boost TriMV transmission by Type 1 mites. Even though the increased TriMV transmission rate for mixed infections was

modest, these findings may help explain why Byamukama *et al.* (2013) found that 91% of TriMV positive samples were co-infected with WSMV. TriMV may be strongly correlated with WSMV because it is benefitted in terms of transmission by co-infected wheat.

WSMV is predominant in field surveys, followed by WMoV and TriMV (Burrows *et al.* 2009, Byamukama *et al.* 2013). Double infections occur at a high rate, most often WMoV and TriMV found with WSMV (Byamukama *et al.* 2013). TriMV can also co-occur with WMoV (Burrows *et al.* 2009). Montana (Type 1) mites increased transmission of WMoV if they were already viruliferous for WSMV (Seifers *et al.* 2002). WSMV has also been shown to increase the transmission rate of TriMV, but with an unknown WCM type (Seifers *et al.* 2009). But these studies focus on WMoV or TriMV transmission in response to WSMV infection and fail to look at WSMV response. The present study is the first to show that WSMV transmission by the WCM is negatively impacted by TriMV presence.

Mite presence data indicate that the reduction in WSMV transmission may be related to mite survival. WCM presence on double infected plants was significantly reduced when compared to WSMV single inoculated plants and the mock treatment but not different than mites feeding on TriMV alone. McMechan (2012) found that WCM feeding on TriMV infected wheat had lower survival and reproductive rates. Siriwetwivat (2006) observed that WSMV enhances WCM reproductive rate of Type 2 WCM.

The exact mechanisms of the interactions between WSMV and TriMV in terms of transmission rates are not known. One explanation might be that co-infection with TriMV

and WSMV increases the concentration of both viruses in susceptible cultivars (Tatineni *et al.* 2010), thus making the virus more readily available for mite acquisition. This information, however, can be dependent on how long the plants have been inoculated. In double inoculations, WSMV concentration decreased at 28 days post inoculation (dpi), when compared to single inoculated plants, while TriMV concentration on double infected plants were still higher than single inoculated plants (Tatineni *et al.* 2010). Stenger *et al.* (2005) found a similar pattern in the co-infection of corn with WSMV and *Maize chlorotic mottle virus* (MCMV). Titers of both viruses were higher in double infected plants at 15 to 17 dpi. But WSMV titers in double infected plants decreased at 28-30 dpi, and were comparable to single inoculated WSMV plants. At 28-30 dpi, double infected plants still had higher MCMV concentration than single inoculated plants (Stenger *et al.* 2007). The WCM fed on the source plants for about two weeks and at the time of single mite transfers, each source plant had been inoculated for at least 16 days or 21 days. Given that Tatineni *et al.* (2010) only analyzed plants at 14 vs. 28 dpi, it might be possible that by the time we picked up the mites for transfers to test plants, WSMV was already decreasing in concentration in double infected plants. But WSMV is retained through molting, so it is also plausible that any given mite used for transmission acquired the virus prior to titers decreasing.

The WCM depends on HC-Pro of WSMV for transmission (Stenger *et al.* 2005). TriMV mechanisms of transmission have not yet been studied. But our results suggest a mechanism in WSMV that facilitates virus acquisition and/or transmission of TriMV by the NE mite.

In summary, we found an increase in TriMV transmission along with reduced WSMV transmission rates using WCM Type 2. When source plants were co-infected with both viruses, single WCMs transmitted TriMV by itself more than it transmitted both viruses and WSMV by itself (Fig.1). Suggesting that not only WSMV enhances TriMV transmission by Type 2 WCM, but also that TriMV interferes with WSMV transmission. The differences in transmission, 14.3 % decrease in WSMV transmission in double inoculated plants and 13.5% increase in transmission of TriMV, were statistically significant, but they are not drastic increases and should be interpreted with caution. In these experiments we are only making inferences about the interaction of WSMV and TriMV on single mite transmission. Additional studies must be conducted to look at WCM population biology and vector capabilities when exposed to WSMV+ TriMV plants.

Future studies should investigate if there are any additional mechanisms behind why TriMV transmission increases while WSMV decreases in double infections. Work in other systems has shown that co-infecting viruses can be antagonistic in a plant and can be segregated in different plant tissues (Dietrich and Maiss 2003, Takahashi *et al.* 2007, Elena 2011). Maybe co-inoculation with WSMV and TriMV can benefit TriMV systemic infection, while inhibiting WSMV infection. Even though Type 1 WCM does not transmit TriMV, we need to know if co-infection of WSMV and TriMV can reduce WSMV transmission rates by the Type 1 mite. More in depth research needs to be performed to determine the implications of these findings.

## Tables

**Table 2.1. Virus transmission rates for Type 2 WCM's feeding on single and in double inoculated plants.**

Treatment	Positive samples/ total	Mean Percentage	Mean S.E.
<b>WSMV transmission</b>			
WSMV	60/120	50.0	5.7
WSMV (+TriMV)	43/120	35.7	5.3
<b>TriMV transmission</b>			
TriMV	51/117	43.3	5.6
TriMV (+ WSMV)	68/120	56.8	5.6

Num df= 1, Den df= 227 for WSMV transmission.

Num df=1, Den df=224 for TriMV transmission.

**Table 2.2. Differences of least square means on Type 2 WCM transmission for single mite transfers.**

Treatment	Treatment	Estimate	Standard error (S.E)	D.F.	t-value	Pr> t	Odds ratio
WSMV+TriMV	WSMV	-0.59	0.265	227	-2.22	0.0027	0.55
WSMV+TriMV	TriMV	0.54	0.265	224	2.04	0.0425	1.72

**Table 2.3. Impact of mite survival post single mite transfers on WSMV transmission rates.**

Treatment	WCM survival	Mite survival	Mean % WSMV	Mean S.E.	t-grouping <sup>1</sup>
Double	37%	Yes	68.3	6.8	a
		No	16.1	4.1	b
WSMV	65%	Yes	59.7	5.4	a
		No	31.8	7.1	b

<sup>1</sup>t-grouping for treatment least square (LS) means. LS-means with same letter are not significant at ( $\alpha=0.05$ ). Num df= 1, Den df= 225.

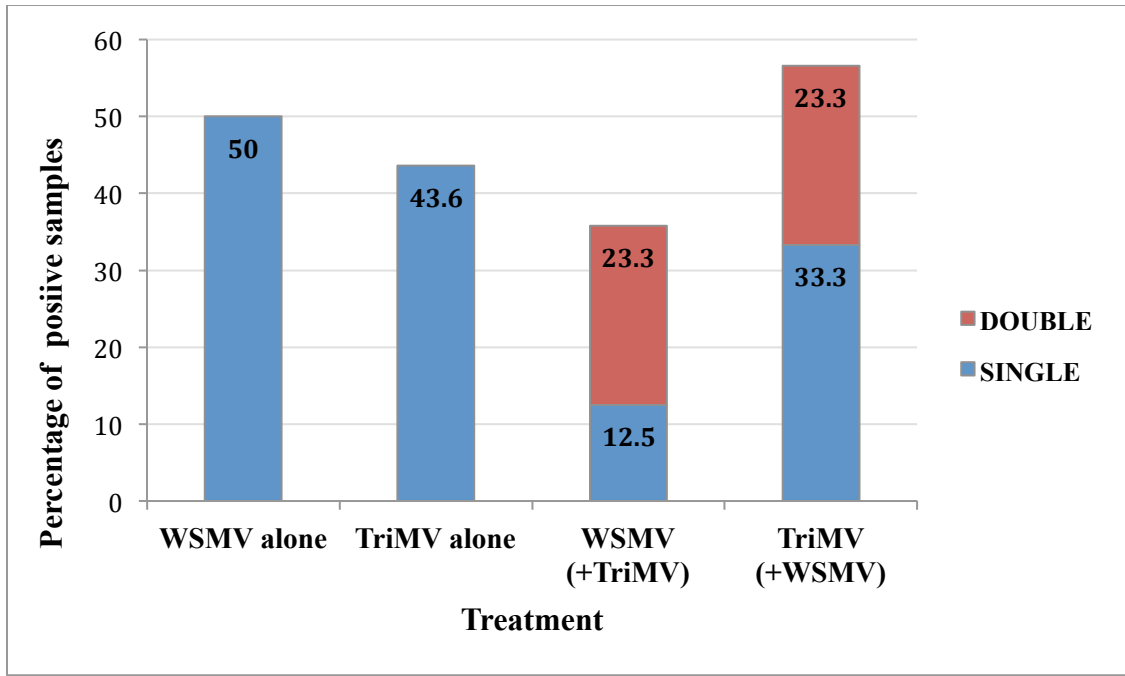
**Table 2.4. WSMV virus transmission in double infected plant by Type 1 WCM (SD, MT) and Type 2 (NE).**

Colony	Positive samples/ total	Mean Percentage	Mean S.E.	t-grouping <sup>1</sup>
NE	44/90	45.5	11.8	a
SD	36/90	36.5	11.1	a
MT	20/80	20.9	8.4	b

<sup>1</sup>t-grouping for treatment least square (LS) means. LS-means with same letter are not significant at ( $\alpha=0.05$ ). Num df=2, Den df=256.

## Figures

Fig 2.1. Percentage of positive virus samples transmitted by Type 2 WCM.





**Chapter 3: Impact of co-infection with *Wheat streak mosaic virus* and  
*Triticum mosaic virus* on the biology of the wheat curl mite and disease  
dispersal in the field**

## Introduction

The wheat curl mite (WCM), *Aceria tosichella* Keifer, is a common pest of wheat (*Triticum aestivum* L.) around the world. WCM infestations on wheat can cause total leaf trapping if plants are young and mild rolling of leaf edges if plants are older (Staples and Allington 1956). Minor losses of wheat can be associated with WCM presence (Harvey *et al.* 2000), but the greatest impact is due to the WCM transmitting viruses to wheat. The WCM is the only known vector of *Wheat streak mosaic virus* (WSMV), wheat mosaic virus (WMoV) and *Triticum mosaic virus* (TriMV) (Slykuis 1955, Seifers *et al.* 1997, Seifers *et al.* 2009). The WCM has been classified into two genotypes, Type 1 and Type 2 (Carew *et al.* 2009, Hein *et al.* 2012). These genotypes vary in transmitting capabilities, but Type 2 mites are able to vector all three viruses (Seifers *et al.* 2002, Siritwetwivat 2002, McMechan 2012).

WSMV (genus *Tritimovirus*, family *Potyviridae*) (Stenger *et al.* 1998) can be transmitted by all stages of the WCM, but the virus cannot be acquired by adult WCM (Orlob 1966). Using the Ng and Falk (2006) classification of vector virus transmission, WSMV would be classified as a persistent-circulative virus since it is retained through WCM's molt. However, it is unknown whether WCM transmission of WSMV requires a latent period, a requirement for persistent-circulative viruses. After acquiring the virus, WCM can be infective for 7 days at 23°C to 28°C and for 61 days at 3°C, but the author noted that infectivity decreases with mite age (Orlob 1966). Using Type 2 WCM, rates of transmission of WSMV on plants with surviving mites after single mite transfer are higher for nymphs (82.8%) and molting mites (94.4%) than for adults (50%)

(Siriwetwivat 2006). Type 2 mites feeding on WSMV infected wheat have significantly higher reproductive potential than mites feeding on virus free plants (Siriwetwivat 2006).

TriMV (genus *Poacevirus*, family *Potyviridae*) (Tatineni *et al.* 2009) was discovered in 2006 in WSMV resistant varieties (Seifers *et al.* 2008). The virus mode of transmission for TriMV is not known, but it is thought to be similar to WSMV. Through single-mite transfer tests, TriMV was transmitted (41%) by Type 2 WCM and can only be transmitted by Type 1 WCM at low rates (2%) under very high populations (McMechan 2012). WCM feeding on TriMV infected plants have their reproductive potential reduced compared to mites feeding on virus free plants (McMechan 2012).

Interactions among WSMV, TriMV, and WMoV can alter transmission rates of each virus. A Montana-collected population (MT) of Type 1 WCM had an increased rate of WMoV transmission if they were viruliferous for WSMV (Seifers *et al.* 2002). Using an unknown mite source, Seifers *et al.* (2009) observed that TriMV transmission rate significantly increased when the source plant was co-infected with WSMV. Our studies (Chapter 2) demonstrated that TriMV has a boost of 13.4% in transmission when the WCM Type 2 feeds on co-infected plants and WSMV has a reduction of 14.3% transmission, compared to mites feeding on single inoculated plants. These studies were performed in the laboratory, but the impact of viral interactions on transmission has yet to be validated in the field. WSMV is the most prevalent virus in this WCM-virus complex and is mostly found alone in samples from the field (Byamukama *et al.* 2013). Double infections of wheat with WCM transmitted viruses are more common, but triple infections can also occur (Burrows *et al.* 2009, Byamukama *et al.* 2013). However,

TriMV appears to be highly dependent on WSMV presence as 91% of TriMV positive samples were found in plants co-infected with WSMV (Byamukama *et al.* 2013).

WSMV and TriMV exhibit disease synergism when they co-infect wheat, by worsening disease symptoms and increasing titers of both viruses in specific cultivars (Tatineni *et al.* 2010). When WSMV and TriMV co-infect wheat they can increase disease severity in both susceptible and resistant cultivars (Byamukama *et al.* 2011, Tatineni *et al.* 2010). The susceptible winter wheat cultivar ‘Millennium’ had fewer tillers and reduced shoot weight in double infection treatments than in the single inoculated treatments (Byamukama *et al.* 2011). In the susceptible cultivars ‘Tomahawk’ and ‘Arapahoe’, WSMV and TriMV co-infection greatly increased virus concentration of both viruses and exacerbated symptoms (Tatineni *et al.* 2010). ‘Mace’, a winter wheat resistant cultivar, at 19°C was resistant to WSMV, and surprisingly, to TriMV as well. But when kept at 20-26°C both WSMV and TriMV were able to replicate and showed mild symptoms (Tatineni *et al.* 2010). Mild symptoms from co-infection of Mace were also noticed by Byamukama *et al.* (2011).

Disease outbreaks are more severe if infection occurs early in the season and in years with warm temperatures (Wegulo *et al.* 2007). Management of the WCM-virus complex relies heavily on the management of over-summering hosts for the WCM (Wegulo *et al.* 2007). The main over-summering host of the WCM and its associated viruses is volunteer wheat that emerges prior to wheat harvest. This volunteer wheat poses a greater risk to fall infestations than post-harvest volunteer, because mites can readily move to the emerging plants to survive the over-summering period (Staples and Allington 1956).

Relatively few studies have been done to observe the dispersal of WCM and/or virus in field situations. Remote sensing was used to estimate WSMV spread in field areas, and consequently determine mite dispersal (Stilwell 2009). Surveys determined the prevalence of WCM transmitted viruses across the Great Plains (Burrows *et al.* 2009, Byamukama *et al.* 2013). But no study has investigated the direct interaction among WCM and its transmitted viruses in field situations. Our field study was designed to replicate the interaction among mite-viruses and volunteer wheat we would find in agricultural fields. The objective of this field study was to determine the impact of varying transmission rates on virus epidemiology under field conditions. We wanted to determine the impact of single and double infections of wheat by WSMV and TriMV on mite populations in the field.

## Materials and Methods

Four virus inoculation treatments were arranged in a randomized complete block design with six blocks in each of two years. Plots were established in block plantings of winter wheat at the Agricultural Research and Development Center near Mead, NE. Inoculations treatments included: mock (water inoculation), TriMV only, WSMV only and WSMV+TriMV.

Inoculations were carried out by placing mite infested plants in the middle of small cage-covered field plots to allow mites to disperse and spread the virus. In 2012, inoculations were carried out on 4-week old 'Millennium' winter wheat on September 12. In 2013, inoculations were carried out on 4-week old winter wheat, cv. 'Settler CL', on June 12. Individual plot area was ca. 60 cm by 60 cm and covered four rows of wheat with row spacing of ca. 18 cm. A 10-cm dia golf cup cutter was used to place a hole in the middle of each plot. A 1 quart cup (11 cm dia, 13 cm deep) was placed into the hole and filled with water. A wood insert (110mm) was placed into the top of the cup to hold a single cone-tainer with the source plant. White polyester cages (60X60X60cm) (Megaview Science Co., Ltd., TW) with 680  $\mu$ m mesh aperture covered the sampling area. Individual plots (cages) were separated by ca. 3-5 m, depending on the availability of good wheat stands.

Source plants for this study were inoculated in the same manner as Chapter 2. In 2012, approximately 50 mites were added to the source plants seven days post inoculation (dpi). The mite populations in the source plants were allowed to build up for seven days and then placed in the field. In 2013, approximately 50 mites were added to the source plants only two days post inoculation to enable plants to be healthier when placed

into the field. After infestation, plants were placed in a growth chamber at 27 °C for nine days and then transferred to the field.

Treatments were monitored weekly to bi-weekly to evaluate the quality of the source plants and virus symptom development of the caged plants in the plot. Symptoms were quantified with the use of a SPAD meter (Konica Minolta Sensing Singapore Pte. Ltd.) relative chlorophyll measurement, resulting in higher values corresponding to greener plants. SPAD readings were taken on 10 plants in each row within the treatment cage, and the values were then averaged and recorded. Five tillers per row for a total of 20 samples per plot were sampled and taken back to the laboratory. From each individual tiller, the number of mites was counted and then tested for the presence of WSMV and TriMV via double-antibody sandwich enzyme linked immunosorbent assay DAS-ELISA (Seifers *et al.* 2009). Tillers were sampled seven weeks after the inoculation plants were added to the plots in 2012 and at both four and six weeks after the inoculation plants were introduced in 2013. Data were analyzed by using SAS 9.2 (PROC GLIMMIX). A binomial distribution was specified for virus presence. Virus incidence was compared for WSMV vs. WSMV+TriMV and TriMV vs. WSMV+TriMV. Mite counts had a negative binomial distribution, and overdispersion of counts was accounted for by using the quadrature method.

## Results

**Field study.** In 2012, the virus infection rate in WSMV inoculated cages was 43.3% (Table 3.1). In cages co-inoculated with WSMV and TriMV, WSMV infection was significantly lower at 24.2% (Table 3.1, and Table 3.2:  $P > |t| = 0.0278$ ). TriMV-only inoculated cages were infected at a rate of 25.5% (Table 3.1). Co-inoculated cages showed a TriMV infection rate of 39.7% (Table 3.1). Despite the fact that co-inoculated cages had higher TriMV infection rate than single infected cages, this difference was not significant ( $P > |t| = 0.3113$ , Table 3.2).

In 2013, we removed our field cages three weeks post field infestation, to prevent the buildup of serious foliar fungal infections. As a result, the TriMV only plots were contaminated by an influx of WSMV viruliferous mites, resulting in a WSMV infection rate of 20%. In addition, there was 17.5% contamination rate of WSMV in the Mock treatment (Table 3.4). No contamination by TriMV was detected in any plots, but the TriMV infection rate increased from 12.5% in the single inoculated to 37.5% ( $P > |t| = 0.0085$ ) in the double inoculated treatment.

Mite counts in 2012 differed among treatments, but were not all significantly different (Table 3.3). WSMV cages had the highest WCM counts averaging 121.1 mites per tiller, followed by mock cages at 66.4 mites per tiller, then the WSMV+TriMV cages at 36.1 mites per tiller, and the TriMV cages at only 14.3 mites per tiller (Table 3.3). WSMV mite densities were significantly higher than WSMV+TriMV and TriMV alone. Mock mite counts are only statistically different than TriMV, and TriMV mite counts are statistically lower than the other three treatments. In 2013 mite population densities were lower, but the pattern among treatments was still the same (Table 3.5). WSMV cages had



a mean of 70.6 WCM per tiller, followed by the mock treatments at 41.1 WCM per tiller, the WSMV+TriMV treatments at 30.6 WCM per tiller and the TriMV treatments at 22.9 WCM per tiller (Table 3.5). WSMV mite counts were significantly higher than WSMV+TriMV and TriMV alone, but these later two treatments are not different from each other. Mock counts were not statistically different than the other three treatments.

Combining the SPAD readings taken on 18 October, 2012 and 2 November, 2012, we had an average value of 51.0 for the mock treatment, 49.7 for TriMV, 47.7 for WSMV and 43.7 for WSMV+TriMV treatment (Fig. 3.1). Mock was significantly higher than WSMV and WSMV+TriMV; however, TriMV was not different than the mock or WSMV treatments (Table 3.6). SPAD readings taken 18 July, 2013 were 34.2 for mock, 31.0 for WSMV, 30.2 for TriMV and 28.6 for WSMV+TriMV (Figure 3.2). Only mock readings were significantly different than the other treatments (Table 3.6).

## Discussion

WSMV and TriMV co-infection increases disease development on susceptible cultivars (Tatineni *et al.* 2010). Our studies determined that the co-infection of wheat by WSMV and TriMV in the field has an impact on disease epidemiology. By the end of the field study in both years we observed lower SPAD readings for double inoculated cages than mock, supporting Byamukama *et al.* (2011, 2014) results. Lower relative chlorophyll readings indicate that viruses were well established in caged treatments.

In 2012, we saw a reduction of WSMV prevalence in cages where TriMV was also present. But in 2013, we had WSMV contamination across the field plots, and as a result, we found similar WSMV infection across all WSMV and WSMV+TriMV inoculated plots (35% vs. 42 %, non significant  $P > 0.05$ ). The 2012 results validate the results seen in the controlled transmission studies done in the lab (see Chapter 2) with reduced WSMV transmission in the presence of TriMV. We are the first to report the negative impact of co-infection on WSMV transmission under laboratory and field conditions.

TriMV infection was higher in double inoculated cages than single inoculated cages, but this difference was not statistically significant ( $P > 0.05$ , 39% vs. 25%). But in 2013, we saw a significant increase in TriMV infection in double inoculated treatments over single inoculated cages (12% vs. 37%). These findings support our TriMV transmission data performed in the laboratory (Chapter 2). The increased TriMV transmission on double inoculated cages help explain why TriMV was found in the field primarily in co-infections with WSMV reported by Byamukama *et al.* (2013).

Previous work has shown that WSMV increases the reproductive potential of Type 2 WCM (Siriwetwivat 2006) and that TriMV decreases their reproductive potential (McMechan 2012). Field data from both years of this study support these findings. We saw the pattern of highest mite counts in the WSMV treatment followed by the mock treatment, then the WSMV+TriMV treatment and the TriMV treatment with the lowest mite presence. In 2013, field contamination from outside mites did occur when the plot cages were removed ca. three weeks after inoculation. This likely occurred at a very low level as only 20% of the tillers showed virus contamination. This low mite contamination rate probably had little impact on the overall mite populations that were measured.

In field studies, we showed that double inoculations reduced WSMV prevalence when compared to single infections. But TriMV infection rate was similar in single and in double infections. Overall, TriMV hindrance of WCM reproduction probably counter balances with the faster reproductive rate found with mites feeding on WSMV. Thus, when mites are exposed to both viruses their reproduction capabilities are only higher than mites feeding on TriMV alone. Future studies should focus on the nature of the co-infections at a cellular level to indicate what might be the cause of our results. Additionally, studies on triple infections of WSMV, WMoV, and TriMV would be necessary to understand more about the WCM disease complex.

## Tables

**Table 3.1. Virus incidence for single and double inoculated treatments in the field experiment, ARDC, Mead, NE 2012.**

Treatments	Number of positive WSMV/ total transmission rate	Mean percentage	Mean S.E.
<b>WSMV incidence</b>			
WSMV	52/120	43.3	11.5
WSMV (+TriMV)	29/120	24.2	4.8
<b>TriMV incidence</b>			
TriMV	34/120	25.5	8.01
TriMV (+WSMV)	48/120	39.7	9.61

Num df=1, Den df=5 for each treatment.

**Table 3.2. Differences of treatment least square means for field experiment 2012.**

Treatment	Treatment	Estimate	Standard error (S.E)	D.F.	t-value	Pr> t	Odds ratio
WSMV+TriMV	WSMV	-0.8753	0.2857	5	-3.07	0.0278	0.417
WSMV+TriMV	TriMV	0.6558	0.5824	5	1.13	0.3113	1.927

**Table 3.3. Treatment mite counts on field experiment 2012.**

Treatment	Mean mite counts	S.E. mite counts	T grouping <sup>1</sup>
WSMV	121.1	27.2	a
Mock	66.4	15.9	ab
WSMV+TriMV	36.1	8.4	b
TriMV	14.3	3.5	c

<sup>1</sup>t-grouping for treatment least square (LS) means. LS-means with same letter are not significant at ( $\alpha=0.05$ ). Num df= 3, Den df= 15.

**Table 3.4. Frequency of positive samples by treatment for field experiment, ARDC, Mead, NE 2013 (collected at 4 weeks).**

WSMV	TriMV	Treatment	Detected/total	Percentage
Negative	Negative	Double	20/40	50
Positive	Negative	Double	5/40	12.5
Negative	Positive	Double	3/40	7.5
Positive	Positive	Double	12/40	30
Negative	Negative	WSMV	26/40	65
Positive	Negative	WSMV	14/40	35
Negative	Negative	TriMV	29/40	72.5
Positive	Negative	TriMV	6/40	15*
Negative	Positive	TriMV	3/40	7.5
Positive	Positive	TriMV	2/40	5*
Positive	Negative	Mock	7/40	17.5*
Negative	Negative	Mock	33/40	82.5

\* Field contaminated by outside mites.

**Table 3.5. Treatment mite counts on field experiment 2013  
(Harvested at 6 weeks).<sup>1</sup>**

Treatment	Mean mite counts	S.E. mite counts	t-grouping <sup>2</sup>
WSMV	70.6	13.3	a
Mock	41.1	7.9	ab
WSMV+TriMV	30.6	6.0	b
TriMV	22.9	4.6	b

<sup>1</sup> 2013 field contaminated by outside viruliferous WCM mites.

<sup>2</sup> t-grouping for treatment least square (LS) means. LS-means with same letter are not significant at ( $\alpha=0.05$ ). Num df=3, Den df=15.

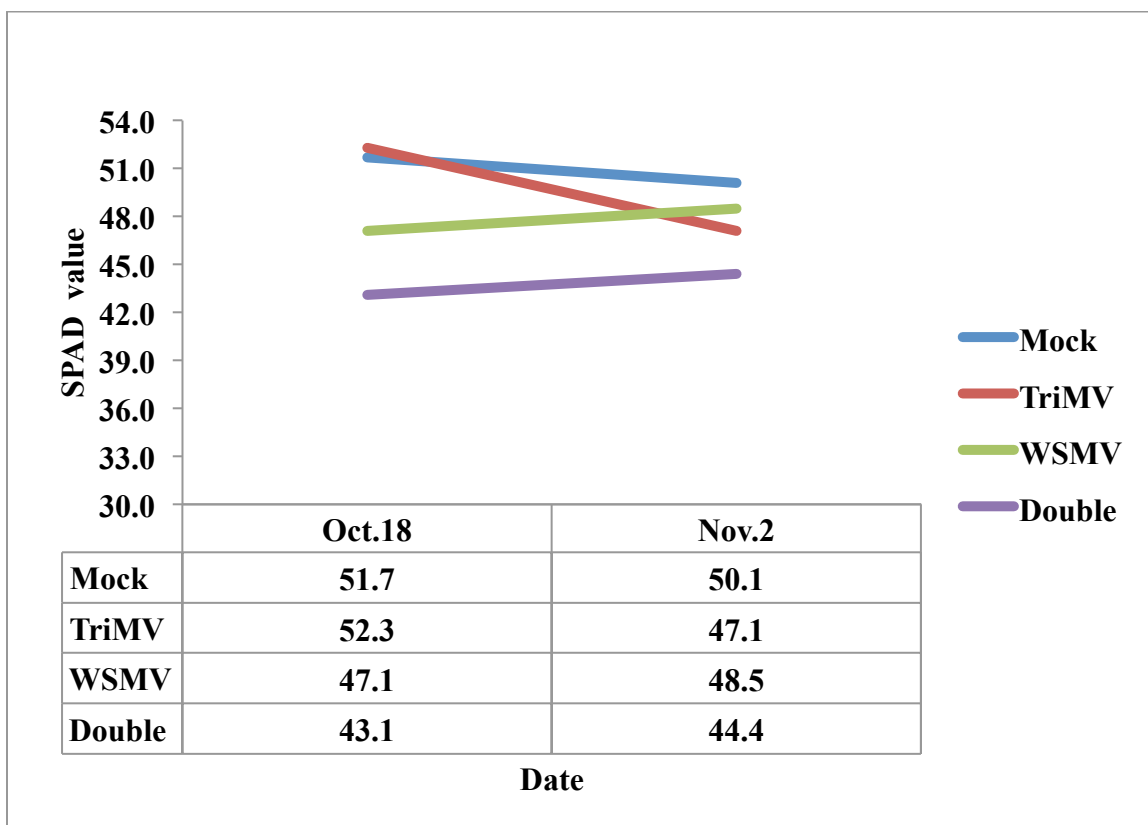
**Table 3.6. Relative chlorophyll (SPAD) reading averages for 2012 and 2013.**

Treatment	Avg. SPAD 2012	t-grouping <sup>1</sup>	Avg. SPAD 2013	t-grouping <sup>1</sup>
Mock	50.9	a	34.2	a
TriMV	49.7	ab	30.1	b
WSMV	47.7	b	31	b
WSMV+TriMV	43.7	c	28.6	b

<sup>1</sup> t-grouping for treatment least square (LS) means. LS-means with same letter are not significant at ( $\alpha=0.05$ ).

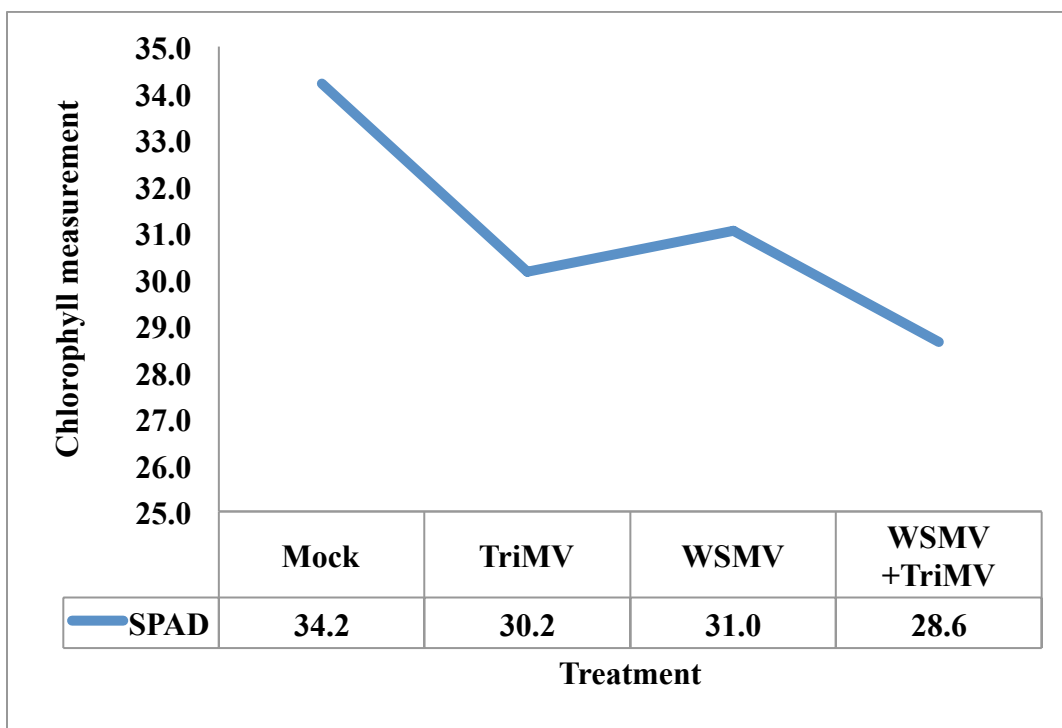
## Figures

**Fig 3.1. Average relative chlorophyll (SPAD) readings for single and double virus-infected field plots on Oct.18 and Nov.2 (5 and 7 weeks post infestation), 2012 field study.**





**Fig 3.2. Average relative chlorophyll (SPAD) readings for single and double virus-infected plots on July 18, 2013 (5 weeks post infestation).**



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