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Transmission of *Triticum mosaic virus* and its Impact on the Biology of the Wheat Curl Mite *Aceria tosichella* Keifer (Eriophyidae), and an Evaluation of Management Tactics for the Wheat Curl Mite and the Wheat-Mite-Virus Complex

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TRANSMISSION OF TRITICUM MOSAIC VIRUS AND ITS IMPACT ON THE

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(ERIOPHYIDAE), AND AN EVALUATION OF MANAGEMENT TACTICS FOR

THE WHEAT CURL MITE AND THE WHEAT-MITE-VIRUS COMPLEX

by

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TRANSMISSION OF TRITICUM MOSAIC VIRUS AND ITS IMPACT ON THE BIOLOGY OF THE WHEAT CURL MITE *ACERIA TOSICHELLA* KEIFER (ERIOPHYIDAE), AND AN EVALUATION OF MANAGEMENT TACTICS FOR THE WHEAT CURL MITE AND THE WHEAT-MITE-VIRUS COMPLEX

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The wheat-mite-virus complex is an important production constraint to winter wheat production in the Great Plains, and consists of three viruses; *wheat streak mosaic* (WSMV), *wheat mosaic* (WMoV) and *Triticum mosaic virus* (TriMV). Synergistic interactions between these viruses have resulted in increased rates of replication and transmission of viruses, thus increasing the potential impact on wheat yields. The wheat curl mite (WCM), *Aceria tosichella* Keifer is the only known vector of the viruses within the wheat-mite-virus complex.

Currently, three colonies of WCM have been characterized by differential responses to mite resistant genes (biotypes) in wheat and differential transmission of WMoV. A study was designed to determine TriMV transmission for these various wheat curl mite colonies. For each source plant, individual mites were transferred to 10 separate test plants and virus transmission determined via ELISA. Results indicate that TriMV is only transmitted by one of the three wheat curl mite colonies using single mite transfers.

An additional study was conducted to determine the impact of TriMV on the biology of the WCM. TriMV infected and uninfected plants were infested with 10 mites from each colony with population counts being taken every seven days. Results indicated that TriMV had a negative impact on the reproductive potential of the WCM. The results demonstrate the importance of the mite source on virus epidemiology.

Management tactics to reduce the impact of the wheat-mite-virus complex have focused primarily on the control of volunteer wheat; however, these tactics are not always effective at reducing yield losses. A field study was conducted from 2007-2011 to determine the impact of the combination of resistant variety and planting date on wheat yields under high virus pressure. Results indicated that both management tactics had a significant impact on yield; however, the combination of tactics provided the greatest yield potential under high virus pressure.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Literature Review

Introduction

The wheat-mite-virus complex is the second largest cause of yield loss in winter wheat production in the Kansas over a 20-year period (Appel *et al.* 2007). Localized yield losses up to 100% are not uncommon (McNeil *et al.* 1996). The complex consists of three viruses; *wheat streak mosaic* (WSMV), *wheat mosaic* (*High plains virus*) (WMoV) and *Triticum mosaic virus* (TriMV). The wheat curl mite (WCM) *Aceria tosichella* Keifer, is the only known vector of the viruses within this complex (Slykhuis 1955; Seifers *et al.* 1997; Seifers *et al.* 2008).

Multiple tactics have been employed over the years to manage the wheat curl mite and the viruses it transmits. Controlling volunteer wheat is critical to managing this complex, however, situations have occurred in the past where management tactics were not conducive to volunteer wheat and significant losses of winter wheat still occurred (Christian and Willis 1993). Resistant wheat varieties have been developed to control the WCM and the viruses it vectors. WCM populations have adapted to mite resistant varieties. These same mite populations have been documented as having different rates of virus transmission (Seifers *et al.* 2002) and reproductive rates (Siriwetwiwat 2006) when associated with the viruses within wheat-mite-virus complex. There is need for a greater understanding of interactions between these organisms and their impact on wheat yields.

Wheat Curl Mite Classification

The WCM is a member of the family Eriophyidae, and it occurs throughout the world (Oldfield and Proeseler 1996). Within North America, the taxonomic history of the principal species of *Aceria* that occurred on cereals is uncertain (Frost and Ridland 1996). North American mites found on wheat were first identified by Keifer in 1938 as the dry bulb mite, *Aceria tulipae* Keifer because of morphological similarities. Keifer believed that the mites found on wheat were the same species of mite infesting tulips (*A. tulipae)*. In 1970, Shevtchenko *et al.* proposed that the specific epithet *A. tulipae* belonged only to mites found on Liliaceae and proposed the name *Aceria tritici* for mites infesting wheat. Prior to this publication, Keifer had described a mite on wheat in Yugoslavia that was identical to *Aceria tritici* as *Aceria tosichella* (Keifer 1969). Because Keifer's publication preceded Shevtchenko's publication, the name *Aceria tosichella* Keifer takes precedence. Keifer's publication resulted in the separation of *A. tulipae* and *A. tosichella* into two distinct species (Amrine and Stasny 1994). Although the distinction between *A. tulipae* and *A. tosichella* was made in 1969, it was not adopted into common use until Armine and Stasny (1994) clarified the historical record. In 1971, Newkirk and Keifer removed mites from *Aceria* and reassigned them to *Eriophyes*, mites in *Eriophyes* were reassigned to *Phytoptus,* and those in *Phytoptus* were assigned to a new genus *Phytocoptella*. Several authors objected to this revision. WCM were restored to the genus *Aceria* in 1989 (Armine and Stasny 1994). As a result, since 1969 the wheat curl mites have been

referred to under multiple species names in the literature including *Aceria tulipae*, *Eriophyes tulipae,* and *Aceria tosichella*.

The complex of viruses WCM transmit is a major cause of loss in winter wheat production in the Great Plains. To reduce economic impact from this complex, varieties with resistance to the WCM were developed. The first mite resistant wheat variety resulting from a translocation from rye was registered in 1987 and deployed as 'TAM 107' (Porter *et al.* 1987). TAM 107 in addition to other varieties with the same gene for resistance to the WCM was adopted and widely distributed throughout the west-central Great Plains during the late 1980's and 1990's. WCM populations that were adapted to TAM 107 were identified in Kansas in the mid-1990's (Harvey *et al.* 1995a; Harvey *et al.* 1997). To determine the extent of this adaptation, Harvey *et al.* (1999) tested WCM from six distinct geographical locations within the Great Plains. Harvey *et al.* (1999) placed these mites on varieties of wheat with different origins of WCM resistance (Harvey and Martin 1992; Thomas and Conner 1986; Whelan and Conner 1989; Cox et al. 1999; Martin *et al.* 1993; Sebesta *et al.* 1994). Results from the study indicated that mites collected from different locations varied in their responses to the different sources of mite resistance (i.e. biotypes).

These same populations were tested for their transmission of WMoV (Seifers *et al.* 2002). Three populations (Kansas, South Dakota and Texas) were inefficient transmitters of WMoV with transmission rates of 1-6%. The Montana population was shown to be intermediate in their transmission rate (15%). Mites in the Nebraska

population were the most efficient transmitter at a rate of 64% using 10 mites per test plant. The Montana population demonstrated an increased transmission rate (52%) when mixed infections of WMoV and WSMV were used.

Hein *et al.* (unpublished) tested these same populations for genetic differences using PCR-RFLP of the mitochondrial cytochrome oxidase subunit I (COI) and cytochrome oxidase subunit II (COII) region and ribosomal DNA. Two distinct populations were identified; type 1 (Kansas, Montana, South Dakota and Texas) and type 2 (Nebraska). The separation between these two types of *A. tosichella* was comparable to their separation with *A. tulipae*, indicating the extent of the differences between the two types. The differences in mite types found within North American mite populations were the same as those found in studies conducted on WCM in Australia (Carew *et al.* 2009).

Viruses Transmitted by the Wheat Curl Mite

WSMV is considered to be the most prevalent of these viruses occurring in part of North America, Europe, the Middle East, North Africa, and Central, East and Southeast Asia (Jones et al. 2005). Annual losses in the Great Plains in North America range from 1% to 5% with localized outbreaks causing yield losses up to 100% (Christian and Willis 1993). Little is known about the epidemiology of WMoV and TriMV. These viruses are often found in combination with WSMV in the field. Studies have indicated that interactions between these viruses can result in increased transmission (WSMV and

WMoV) (Seifers *et al.* 2002) or increased yield impacts on wheat (WSMV and TriMV) (Tatineni *et al.* 2010; Byamukama *et al.* 2012).

Wheat Streak Mosaic Virus

Wheat streak mosaic virus (WSMV) was identified in 1922 as 'yellow mosaic' by Peltier (Staples and Allington 1956). It is the type species of the genus *Tritimovirus* in the family *Potyviridae* (Stenger *et al.* 1998). WSMV is a single stranded RNA virus with ~9384 nucleotides and is translated as a single polyprotein (Choi *et al.* 2002). WSMV has distinct resident populations in North America and Eurasia (Rabenstein *et al.* 2002). McNeil *et al.* (1996) identified a total of 32 distinct RFLP types in five Nebraska counties. The genetic diversity of these RFLP types was greatest among fields rather than between counties. Although the genetic diversity of populations changed over time they remained geographically homogeneous. This indicates that there was extensive mixing of WSMV isolates.

Three WSMV strains within North America have been completely sequenced (Choi *et al.* 2001). The Type and Sidney 81 strains of WSMV were isolated from wheat in the Great Plains and share 97.6% of their nucleotide sequence identity. Sidney 81 is considered to be the most dominant strain within the Great Plains. In the central highlands of Mexico, the El Batàn 3 strain was isolated from wheat (Sanchez-Sanchez *et al.* 2001). It shares only 79% of its nucleotide sequence with the two strains isolated from

the Great Plains (Choi *et al.* 2001). All three of these strains are vectored by the WCM (Brakke 1958; Choi *et al.* 1999; Sanchez-Sanchez *et al.* 2001; Hall *et al.* 2001).

WSMV is only transmitted by the wheat curl mite; however, there are some indications that the virus can be transmitted via seed at low levels (ca. 0.5% - 1.5%; Jones *et al.* 2005). The discovery of WSMV in Australia was hypothesized to occur through the introduction of wheat breeding seed from the United States (Dwyer *et al.* 2007).

WSMV has a wide host range and can infect many plants within the grass family (McNeil *et al.* 1996). It can infect almost all varieties of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.) (Brakke 1971). Sidney 81 and Type strains can be distinguished from one another based on their virulence to the maize inbred line SDP2 (Choi *et al.* 1999).

Wheat Mosaic Virus

Wheat mosaic virus (WMoV) (genus *Emaravirus,* family Bunyaviridae) was first identified in corn in 1993 (Jensen et al. 1996; Gavin *et al.* 2012). WMoV formerly known as *High plains virus* is a segmented, negative-strand RNA virus associated with a 32-kDa protein, double membrane virus-like particles of 80-200 nm in diameter (Ahn *et al.* 1996). The economic losses associated with WMoV are unknown, but it has a host range consisting of many economically important plants, including wheat and maize (Skare *et al.* 2006). Field samples that tested positive for WMoV often had WSMV. These co-infections often have higher symptomatic expression. WMoV cannot be mechanically

transmitted, but it can be transmitted by vascular puncture through the inoculation of corn seeds (Jensen et al. 1996, Louie and Seifers 1996).

WMoV exhibits different rates of transmission depending on the mite source. Nebraska (Type 2) and Montana (Type 1) mites were able to transmit all five WMoV isolates, whereas Kansas (Type 1) mites transmits only one isolate of WMoV (Seifers *et al.* 2002), albeit poorly. Montana mites that were virulent for WSMV exhibited higher rates of transmission than avirulent mites with just WMoV.

Only a partial host range of WMoV is currently available because WMoV is not mechanically transmissible. Cheatgrass, corn, barley, oats, rye, green foxtail, yellow foxtail, and wheat are susceptible to WMoV (Seifers et al. 1998). To cause infection, high numbers of WCM had to be transferred to cheatgrass, oats, and rye. WMoV can be separated from WSMV and TriMV through mite transmission onto yellow foxtail plants, because WMoV is the only virus capable of infection of this host (Seifers et al. 1998; Skare et al. 2003).

Triticum Mosaic Virus

Triticum mosaic virus (TriMV) (genus *Poacevirus,* family *Potyviridae*) was first identified in wheat in Kansas in 2006 with symptoms almost identical to WSMV (Seifers *et al.* 2008). Wheat plants infected with TriMV were not geographically localized and were often found in combination with WSMV. The wheat curl mite was identified as the vector of TriMV with a transmission rate of 1.3% using single mite transfers (Seifers *et*

al. 2009). TriMV has been identified as a single-stranded RNA virus consisting of 10,266 nucleotides with a polyprotein made up of 3,112 amino acids (Tatineni *et al.* 2009). It is the type member of a new genus *Poacevirus* sharing 49% of its coat protein with *Sugarcane streak mosaic virus* (SCSMV) (Fellers *et al.* 2009; Tatineni *et al.* 2009). TriMV shares only 23.2% of its identity with WSMV (Fellers *et al.* 2009; Tatineni *et al.* 2009). Although TriMV has been identified as a mite vectored virus and should belong to the genus *Tritimovirus* it is significantly divergent enough to be placed in a new genus (Fellers *et al.* 2009; Tatineni *et al.* 2009). Virion morphology and sequence alignments suggest that TriMV did not originate as recombinants or selection from other viral populations (Fellers et al. 2009; Tatineni *et al.* 2009).

TriMV has been discovered in Colorado, Kansas, Nebraska, Oklahoma, South Dakota, Texas and Wyoming (Burrows *et al.* 2009). A survey of symptomatic plants collected in the Great Plains region in 2008 indicated that TriMV was positive in 17% of the samples (Burrows *et al.* 2009). The percentage of positive samples ranged from 57% in Texas to 0% in Montana and North Dakota. TriMV has been shown to impact wheat through reduction in wheat yields and volume weight, but the effect may be cultivar specific (Seifers *et al.* 2011). Tatineni *et al.* (2010) showed that TriMV is synergistic with WSMV in co-infections with TriMV exceeding the titer of WSMV late in the infection process. Greenhouse studies conducted by Byamukama *et al.* (2012) demonstrated that WSMV and TriMV had a negative impact on yield determinants (biomass, tillers, total

nitrogen, and total carbon). It was also shown that these effects were more pronounced on the susceptible variety 'Millennium' when compared with the resistant variety 'Mace'.

The host range of TriMV through mechanical inoculation (Seifers *et al.* 2009; Tatineni *et al.* 2010). Crops identified as susceptible to TriMV were wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), rye (*Secale cereale* L.), and triticale (*Triticosecale rimpaui* Wittm.) while sorghum (*Sorghum bicolor* (L.) and maize (*Zea* mays L.) were not. Some varieties of barley and triticale were susceptible to TriMV but not WSMV. Several grass species were susceptible; including jointed goatgrass (*Aegilops cylindria* Host.), wild oat (*Avena fatua* L.), cheatgrass (*Bromus secalinus* L.), field brome (*Bromus arvensis* L.), prairie cupgrass (*Eriochloa contracta* Hitchc.), tapertip cupgrass (*Eriochloa acuminate* (J. Presl.) Kunth), and green foxtail (*Setaria viridis* L.).

Virus Transmission

WSMV is non-transovarial but has been shown to be transtadial in WCM (Siriwetwiwat 2006). WCM begin acquiring the virus within 15-20 minutes with a transmission rate of <1% (Orlob 1966). When WCM are given a period of 16 hours for acquisition of the WSMV they were able to transmit at a rate of 50%. The acquisition phase was similar to the time required for inoculation (Orlob 1966). WSMV has been detected in the body fluids and gut of the WCM (Sinha and Paliwal 1976; Slykhuis 1967; Stein –Margolina *et al.* 1969; Paliwal 1980). Large numbers of WSMV were found in

the midgut that remained undegraded for at least 5 days. WSMV particles were also discovered in the salivary glands of *A. tosichella* reared on virus infected plants, but the study couldn't be replicated (Paliwal 1980). These findings provide the strongest evidence to date that WSMV is circulated through various body tissues and eventually inoculated through the saliva (Paliwal 1980). Although there is evidence for this type of transmission, regurgitation cannot be ruled out.

Adult WCM must acquire WSMV as an immature in order to transmit the virus (Slykhuis 1955; del Rosario and Sill 1965; Orlob 1966). Orlob (1966) demonstrated that adult WCM could acquire WSMV but was unable to transmit the virus. This was determined by mechanically inoculating plants using macerated WCM that had fed on virus infected plants only after reaching the adult stage. WCM transmit in a semi-persistent manner of transmission because the efficiency of the transmission increases with increased feeding time. Once mites have acquired the virus they can continue to transmit it for at least 7 days at room temperature, and up to 61 days when kept at 3°C (Slykhuis 1955; del Rosario and Sill 1965; Orlob 1966).

Wheat Curl Mite Biology and Ecology

Wheat curl mites (WCM) are white in color with a cigar-shaped body and range in length from 170-250 microns (Keifer 1938). Their small size makes them difficult to see with the naked eye; however, when they accumulate on plants and in mass they can give the impression of a powdery mildew infection (Staples and Allington 1956). Wheat

plants that are heavily infested with WCM often display various degrees of chlorosis. Symptomology of mite infestations can be more severe when plants are under drought conditions (Staples and Allington 1956).

The complete life cycle of the WCM requires 7–10 days, with an egg, larva, nymph, and adult stage (Staples and Allington 1956). Eggs take approximately 4 days to hatch at 25°C. Temperature and humidity are critical to egg hatch. The majority of eggs hatch at 25°C with a relative humidity of 100% (Slykhuis 1955). Egg hatch is almost completely arrested below 15°C (Slykhuis 1955). Humidity is critical to egg hatch. Very few eggs hatched at a humidity of 75%, and no eggs hatching at a relative humidity below 50% due to desiccation (Slykhuis 1955). Each immature stage is approximately 36 hours in length at 25°C. Between each of the stages there is a quiescent phase where the mites remain inactive and appear partially translucent, lasting about 18 hours (Staples and Allington 1956). After an adult emerges, it requires an additional 1-2 day preoviposition period. There are no studies indicating the lifespan of an adult, but it is estimated that adults can live for 20-30 days under ideal conditions. WCM can survive without a host for approximately 48 hours depending on the temperature and humidity.

There are some subtle morphological differences between the growth stages of WCM (Slykhuis 1956). In the larval stage, seta located just behind the head face anterior; whereas, in the nymphal and adult stages, these setae face towards the posterior end. The external reproductive structures only become visible in the adult stage where they appear on the dorsal side towards the anterior end. The genital flap can be used to distinguish

females from males with the use of a microscope. In females the genital flap opens towards the posterior end of the body whereas in males the flap is less pronounced and appears to open anteriorly (Lindquist *et al.* 1996).

WCM have an indirect method of sperm transfer (i.e. no copulation occurs). Males deposit spermatophores on the leaf surface and females later locate and pick them up (Oldfield 1970). The mites are haplodiploid and produce males via arrhenotokous parthenogenesis resulting in haploid males. Fertilized females are capable of producing diploid females and haploid males (Helle and Wysoki 1983). When these males emerge and reach their reproductive stage, they will produce spermatophores to enable fertilization of the female. A female can lay approximately 12-20 eggs during its lifetime. It has been estimated that under ideal conditions that a single female can result in 3 million mites in 60 days. Optimum reproduction for WCM occurs between 23-27°C (del Rosario and Sill 1959). Reproduction slows at 9°C and stops at 0°C (Staples and Allington 1956).

Mite Movement

Nault and Styler (1969) proposed that significant mite movement occurred only when wheat heads and flag leaves were drying out. Greenhouse studies conducted by Thomas and Hein (2003) showed no correlation between mite movement and plant condition. The study indicated a significant correlation between mite population and mite movement. Healthy host plants supported larger mite populations than deteriorating host

plants. Field studies confirmed that healthier hosts supported larger mite populations and as a result, increased mite movement.

WCM move passively between plants and fields via wind dispersal (Sabelis and Bruin 1996). Only adult WCM exhibit dispersal behavior (Nault and Styler 1969). To disperse from plants, adults move to the upper margins of the leaf. At this point they hold their bodies perpendicular to the leaf surface by adhering themselves to the leaf using their caudal sucker. This position raises the mite out of the laminar layer of the leaf surface to areas where wind speeds are exponentially higher (Sabelis and Bruin 1996). When plants are heavily infested, mites can crawl on one another forming chains through the attachment of their caudal suckers (Nault and Styler 1969). Air movement can stimulate perpendicular standing of WCM and the formation of WCM chains. After dispersing from the host it is estimated that less than 10% of mites will reach their primary host again (Jeppson *et al.* 1975).

To avoid desiccation, mites migrate to the inner whorl of a newly emerging leaf shortly after landing on a new host. There they feed between the veins of the plant on a thin epidermal layer of tissue known as the bulliform cell. These cells are important in the unrolling of the leaf as it emerges (Esau 1953). WCM feeding prevents the leaf from uncurling, causing subsequent leaves to become trapped. The curled leaf provides an ideal environment for mite survival. WCM will continue to feed on the leaves, migrating to each newly emerging leaf. Mites also colonize the wheat head as it emerges. Within

the wheat head mites live in secluded sites and feed inside the glumes (Kantack and Knutson 1954).

WCM Alternative Hosts

The primary host plant for the WCM is wheat. Other crops have been shown to host WCM, such as, barley, corn, foxtail millet (*Seteria italica* (L.) P. Beauv.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), oats, sorghum, and rye. Corn has been considered the second most important host to volunteer wheat for oversummering survival of WCM. Nault and Styler (1969) noted that mite populations occurred on corn beginning in mid-August. By late-August and early-September mite populations were estimated at 900 to 1,000 mites per plant. Mite populations on corn steadily declined through late-October. The occurrence of mites on corn can overlap the period between harvest and fall planting of winter wheat, making it a significant alternative host for WCM.

WCM have been shown to reproduce on many wild grasses under greenhouse conditions, including barnyardgrass (*Echinachloa crus-galli* (L.) P. Beauv), jointed goatgrass, cheatgrass (*Bromus tectorum* (L.), western wheatgrass (*Agropyron smithii* Rydb.), tall oatgrass (*Arrhenatherum elatius* L.), grama (*Bouteloua sp.*), smooth brome (*Bromus inermis* Leyss.), sandbur (*Cenchrus pauciflorus* Benth.), smooth crabgrass (*Digitaria ischaemum* (Schreb.)), crabgrass (*Digitaria sanguinalis* (L.)), stinkgrass

(*Eragrostis cilianensis* (All.)), Canada wildrye (*Elymus canadensis* L.), witchgrass (*Panicum capillare* L.), green foxtail, and yellow foxtail (*Seteria glauca* (L.) P. Beauv.) (Connin 1956b; del Rosario and Sill 1965; Wegulo *et al.* 2008).

The value of alternative hosts for WCM is uncertain. Mite reproduction studies on alternative hosts have not been consistent. Connin (1956a) observed no increase in mites on green foxtail; however, del Rosario and Sill (1965) reported that WCM adapted easily to green foxtail. These host range studies were primarily qualitative evaluations. Future studies are needed to provide quantitative data to determine the value and significance of these alternative hosts for the WCM.

Biological strains of mites are suggested to exist (Gibson 1957). WCM occurring naturally or raised on alternative hosts, such as western wheat grass, had difficultly establishing and reproducing on wheat (Gibson 1957). Del Rosario and Sill (1965) attempted to transfer mites from naturally occurring foxtail barley, Canada wild rye, and western wheat grass, but they were unable to survive when placed on wheat plants.

During the 1988 growing season in Kansas a high incidence of WSMV was reported with a low incidence of volunteer wheat in the area (Christian and Willis 1993). This situation suggested the possibility that alternative hosts may serve a significant role as hosts for WCM and WSMV. Green foxtail, giant foxtail, prairie cupgrass, barnyardgrass, and common witchgrass were able to support WSMV during the period between harvest and the new wheat crop emerging in the fall. Current research suggests

that these secondary hosts could serve as an important source of genetic variation for populations of wheat curl mites as well as the viruses they transmit. It is less likely that the hosts will provide a source of mites necessary for widespread epidemic, but if mite populations are given the opportunity to build up they could result in local outbreaks. Research is needed to determine the impact of these alternative hosts.

Impact of Virus Complex and Wheat Curl Mite

Wheat plants infected with virus often show a yellow mosaic pattern of parallel discontinuous streaks (Wegulo *et al.* 2008). As the virus progresses, leaves become mottled yellow. Late stages of symptoms can often be confused with *Barley yellow dwarf virus* (BYDV). BYDV symptoms usually start at the tip of wheat leaves and expand towards the middle and base of the leaf. WSMV infected plants usually remain mottled yellow throughout the whole leaf (Wegulo *et al.* 2008). As WSMV progresses the entire leaf will become pale-yellow similar to that of BYDV but its symptomatic origin is not the leaf tip.

The impact of the virus on plant symptomology also depends on the plant stage when wheat is infected. Wheat infected early in its development (early tillering stage) can become stunted, discolored and rosetted (Wegulo *et al.* 2008). Infections that occur after wheat is well tillered are often not as severe. The extent of symptoms in the field can be a good indication of the severity and yield loss.

WCM feeding causes rolling and trapping of wheat leaves. Leaves infested with WCM often remain erect with the edges of the leaves rolled inward towards the mid-rib. As new leaves emerge they can become trapped in the lower leaf, forming a loop. Trapping of wheat leaves can be a good indication of mite presence in volunteer wheat (Wegulo *et al.* 2008). Leaf trapping can also cause grain heads to become trapped as they emerge (Somsen and Sill 1970).

The impact of viruses transmitted by WCM depends on the time of infection and the density of the mite populations (Wegulo *et al.* 2008). Wheat plants inoculated with viruses early in the fall are at a higher risk for yield loss (Hunger *et al.* 1992). Warmer fall temperatures increase the duration of activity for WCM and may increase their secondary spread. Warmer temperatures also increase virus reproduction and titer in virus-infected plants causing an increase in damage potential. Wheat plants inoculated with WSMV and held at 28°C showed symptoms at 5 days whereas plants held at 15°C required 15 days for expression (Sill and Fellows 1953).

Avirulent or non-viruliferous WCM have been shown to cause yield losses between 1-15% in artificially infested field studies (Harvey *et al.* 2000). In this study, plots were artificially infested with WCM from the greenhouse and averaged an estimated 8,821±3,814 mites/head resulting in a 17% yield loss when compared to naturally infested plots. Mite populations do not normally reach these levels under natural field conditions. A study conducted by Mahmood *et al.* (1998) indicated that randomly selected heads from wheat field averaged around 1,203 mites/head in 1995 and 487

mites/head in 1996 (Mahmood *et al.* 1998). Samples in the study ranged from 3 to 2,958 mites/head. An outbreak in 1988 showed that mites could get as high as 18,000 mites/head (Harvey *et al.* 1990). These events are uncommon and localized, indicating that avirulent WCM have a limited capacity to cause significant yield loss in wheat.

Management of Wheat Curl Mite and Wheat Streak Complex

Volunteer wheat is critical to the oversummering survival of the mites and the viruses it transmits. As winter wheat matures the WCM must find a living host to survive on during the summer (Connin 1956a). Volunteer wheat emerging prior to harvest provides the necessary "green bridge" for mites and virus to survive on during the summer until new wheat emerges in the fall (Wegulo *et al.* 2008). Pre-harvest volunteer wheat is most often caused by hail storms occurring prior to the harvest (Staples and Allington 1956). Hail stones knock seeds from the wheat heads to the soil where they germinate quickly. As the wheat matures and dries, the mites move to the newly emerging volunteer wheat. Widespread virus outbreaks are often linked to volunteer wheat that emerges prior to harvest. Volunteer wheat that emerges after harvest is a much lower risk for over-summering mites and virus. Controlling volunteer wheat eliminates the "green bridge" and prevents large numbers of wheat curl mites from infesting newly emerging wheat in the fall. The most effective management strategy for controlling WCM and their viruses is the control of volunteer wheat.

Some perennial and annual grasses have been shown to support WCM and the viruses they vector (Staples and Allington 1956). Controlling these alternative hosts is not warranted, as they are not important to the epidemiology of WSMV. Other management strategies such as adjusting planting date and growing mite or virus resistant varieties (eg. Mace) have been shown to be effective management practices when the risk of mites and virus is high.

Chemical Control

Use of acaricides for mite control is limited. Kantuck and Knutson (1958) tested over 30 different insecticides on wheat curl mites including many systemic insecticides but had little control without damaging plant health. The high rate of mite reproduction allows populations to respond quickly following an application, if any individuals survive. Mite transmission of plant viruses also limits the effectiveness of acaricides because viruses transmitted by the mites will continue to cause economic damage even if the mites are no longer present. Most importantly, the secluded location of WCM limits effective acaricides to those that are systemic within the plant. Harvey *et al.* (1979) tested the efficacy of systemic carbofuran (FMC Corporation, Philadelphia, Pennsylvania) and disulfoton (Chemagro, Kansas City Missouri) applied to the soil at planting time. Carbofuran controlled mites during the fall, but it lost its efficacy by spring. However; it was shown to increase wheat yields. Carbofuran is one of the most toxic carbamate pesticides, marketed under the name Furadan. It has been recently cancelled due to its high dietary, worker and ecological risks (EPA 2011).

Cultural Control

The most effective management tactic for the control of WCM and its virus complex is the control of pre-harvest volunteer wheat. Controlling volunteer wheat using herbicides can be an effective management tactic. Herbicides such as paraquat (Zeneca Ag Products, Wilmington, Delware) and glyphosate (Monsanto, St. Louis, Missouri) can be used to destroy the "green bridge" host, diminishing the ability for mites to survive through the summer (Jiang *et al.* 2005). Paraquat acted rapidly to reduce mite populations, with effects occurring within a few days. Glyphosate was slower than paraquat, but it may be a better option for producers because of its low toxicity to other non-targets (Jiang *et al.* 2005). Thomas and Hein (2003) indicated that mite movement peaked seven days after a high rate glyphosate treatment. Tillage is also an effective means of controlling volunteer wheat, but it may be less practical in areas where water is limited (Thomas *et al.* 2004). In dry years, wheat yields in no-tillage systems were 72% to 100% higher than fall chisel plowing and conventional tillage, respectively (Bouzza 1990). Tillage was found to be more effective in controlling mite populations on volunteer wheat than glyphosate (Jiang *et al.* 2005). Controlling perennial and native grasses is not warranted because they are not likely to allow mite populations to build up in high enough numbers to cause widespread damage (Staples and Allington 1956).

Another method of managing the wheat curl mite and the viruses it transmits is adjusting the planting date of winter wheat. The earlier wheat is planted in the fall the more likely it is to become infested with mites (Wegulo *et al.* 2008). Planting winter

wheat later reduces the time that mites have to build up and reduces time for virus replication. In addition, it reduces the chance for secondary spread of mites within a field. Temperature is an important consideration when planting winter wheat. If temperatures remain warm in the fall and through the winter the wheat may become infested regardless (Staples and Allington 1956). If wheat is planted too late in the fall then yields may be lower due to agronomic concerns. Hunger *et al.* (1992) found that planting late in the fall was the best method to avoid WSMV; however, planting late made the wheat in the spring more susceptible to WSMV because of its reduced growth.

Host Plant Resistance

Host plant resistance has been developed against the WCM and the viruses it vectors. Wheat resistance to the WCM has been accomplished through reduced reproduction and colonization of the WCM. TAM 107 developed from rye was the first commercial wheat variety with resistance to WCM colonization (Sebesta and Wood 1978; Thomas and Conner 1986). TAM 107 was released in the late-1980's and was widely grown throughout western Kansas and surrounding states. The variety significantly lowered mite populations in wheat spikes and had a lower incidence of WSMV than any other variety at the time (Harvey *et al.* 1998). TAM 107 was critical in preventing WCM build up in volunteer wheat. Widespread popularity of TAM 107 resulted in strains of WCM that were adapted to the mite resistant wheat varieties (Harvey *et al.* 1995; Harvey *et al.* 1997).

Host plant resistance has also focused on resistance to WSMV. There are currently two known sources of resistance that have been transferred to wheat (Huangjun *et al.* 2011). The *Wsm1* gene was transferred from intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth and D. R. Dewey) and confers resistance to WSMV (Friebe *et al.* 1991; Gill *et al.* 1995; Wells *et al.* 1973; Well *et al.* 1982). The *Wsm2* gene, was identified in CO960293-2 wheat germplasm and incorporated into 'RonL' (Seifers *et al.* 2007) and 'Snowmass' (Haley *et al.* 2002). The exact origin of CO960293-2 is unknown because both parents exhibited resistance in greenhouse and growth chamber conditions (Haley *et al.* 2002; Seifers *et al.* 2006). Both sources of resistance are temperature sensitive, becoming ineffective at temperatures above 24°C (Seifers *et al.* 2006). These lines are considered to be valuable sources of resistance in areas where temperatures are cool following planting in the fall (Seifers *et al.* 2006).

Mace released in 2007 is a hard red winter wheat variety adapted to rain-fed and irrigated wheat in Nebraska and areas in the northern Great Plains (Graybosch *et al.* 2009). WSMV resistance in Mace is conditioned by the *Wsm1* gene. Divis *et al.* (2006) concluded that there were no negative effects associated with the *Wsm*1 gene. Graybosch *et al.* (2009) tested Mace for its ability to compete with other wheat varieties. Under virus free conditions Mace was comparable to Millennium. Under natural virus conditions Mace yielded significantly more than Millennium and twice the yield of a highly susceptible variety Tomahawk. Mace is not effective against viruses transmitted by the WCM at temperatures above 25°C (Graybosch et al. 2009). Although Mace was released

for resistance to WSMV, it has also shown resistance to TriMV (Tatineni *et al.* 2010; Byamukama *et al.* 2012).

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CHAPTER 2

Transmission efficiency of Triticum mosaic virus across Wheat curl mite *Aceria tosichella* Keifer (Eriophyidae) colonies collected within the Great Plains

Introduction

The wheat-mite-virus complex is the second largest cause of loss in winter wheat (*Triticum aestivum* L.) production in Kansas over a 20-year period (Appel *et al.* 2007). The wheat curl mite (WCM), *Aceria tosichella* Keifer is the only known vector of the three viruses that make up this wheat-mite-virus complex: *Wheat streak mosaic virus* (WSMV), *Wheat mosaic virus* (WMoV) and *Triticum mosaic virus* (TriMV). WSMV was identified in 1922 as 'yellow mosaic', and it is the most well understood of the viruses within this complex (Staples and Allington 1956). WMoV was identified in the 1990's, and it has been difficult to study because it is not mechanically transmitted (Jensen and Hall 1995). TriMV was only recently identified in wheat in Kansas in 2006 (Seifers *et al.* 2008).

WCM are cigar-shaped, light yellow, and approximately 150-270 microns in length (Staples and Allington 1956). Their complete lifecycle is about 7-10 days, developing from an egg through two immature stages to an adult (Staples and Allington 1956). WCM reproduce by arrhenotokous parthenogenesis, and it is estimated that one female under ideal conditions can result in 3 million mites after 60 days. Average annual losses associated with WSMV are estimated at 5%, however, localized yield losses approaching 100% are not uncommon (McNeil *et al.* 1996).

To reduce the impact of the wheat-mite-virus complex, wheat varieties have been developed with resistance for both the mite and the viruses they transmit. 'TAM 107' was the first commercial variety to be released in the late-1980's with resistance to WCM

colonization (Sebesta and Wood 1978; Thomas and Conner 1986). The popularity and wide spread distribution of this variety led to the development of WCM populations that were adapted to the mite resistant genes (Harvey *et al.* 1995a; Harvey *et al.* 1995b; Harvey *et al.* 1999). Harvey *et al.* (1999) found WCM survival responses to resistant varieties were different depending on the geographic location of the mite collections.

Hein (unpublished) tested these same populations for genetic differences using PCR-RFLP of the COI and COII of the mitochondrial and ribosomal DNA. Results from the study indicated that two distinct mite genotypes existed. 'Nebraska' (Type 2) was genetically distinct from 'Kansas', 'Montana', 'South Dakota' and 'Texas' (Type 1). The magnitude of differences between these two types were comparable to the differences between *A. tosichella* and the dry bulb mite *Aceria tulipae.* Previously, Carew *et al.* (2009) had identified two distinct lineages of WCM in Australia based on a mitochondrial 16S rDNA gene and two nuclear markers. Schiffer *et al.* (2009) tested Carew *et al.* (2009) mite types for transmission of WSMV and determined that only one type was able to transmit the virus. However, North American mite types were found to have no significant differences in transmission of WSMV (Seifers *et al.* 2002). Seifers *et al.* (2002) observed significant differences in transmission of WMoV between the North American mite types. 'Kansas', 'South Dakota' and 'Texas' (Type 1) mites were inefficient transmitters of WMoV at a rate of 1-6%. 'Montana' mites (Type 1) exhibited intermediate transmission at 15%. 'Nebraska' (Type 2) was the most efficient vector,

transmitting at a rate of 64% using 10 mites per test plants. Transmission rates of WMoV increased to 52% for Montana mites when mites were viruliferous for WSMV.

The transmission of WSMV by the WCM has been studied extensively. WSMV is transmitted by all stages of the WCM except the egg stage (Slykhuis 1955; del Rosario and Sill 1965; Orlob 1966; Paliwal and Slykhuis 1967; Siriwetwiwat 2006). Adult WCM are unable to acquire the virus, but they can transmit if the virus is acquired during the earlier stages of development. Adults were less efficient vectors of WSMV when compared with nymphs (Orlob 1966). WCM exhibit semi-persistent transmission with 1% of WCM acquiring virus within 15 minutes of feeding. Mites are capable of transmitting viruses many days after acquisition.

Little is known about the transmission of TriMV. Wheat plants infected with TriMV at that time were not geographically localized and were often found in combination with WSMV. TriMV is a single stranded mRNA consisting of 10,266 nucleotides with a polyprotein of 3,112 peptides and has been placed in the family *Potyviridae* (Fellers *et al.* 2009; Tatineni *et al.* 2009). It is a type member of a newly proposed genus *Poacevirus* sharing 49% of its coat protein sequences with *Sugarcane streak mosaic virus* (SCSMV). TriMV shares only 23.2% of its identity with WSMV, a member of the genus *Tritimovirus*.

Transmission efficiency can affect the rate of spread of a virus and has implications for future studies that involve WCM as vectors. After the discovery of TriMV in Kansas in 2006, an initial transmission rate was established at 2% but the

WCM type was unknown (Seifers et al. 2009). Given the differences in transmission between mite types for WMoV it is important to determine if similar differences exist for the transmission of TriMV. The primary objective of this research was to determine TriMV transmission efficiency by the different wheat curl mite populations collected in the Great Plains region.

Materials and Methods

Three WCM colonies were used in this study, designated 'Nebraska', 'Montana' and 'South Dakota'. These were the same colonies used by Harvey *et al.* (1999) to evaluate mite resistance to WCM resistant wheat varieties, Seifers *et al.* (2002) to evaluate the transmission of WMoV, and Hein (unpublished) to characterize wheat curl mites by PCR-RFLP. WCM colonies used in this study were maintained on 'Millennium' wheat which was caged from the time it was planted. Cages were made of plastic sheeting molded together to form a 15-cm-diameter cylinder. Two, 8-cm diameter holes were cut on opposite sides of the cage approximately 1/3 of the way up the cage. The top of the cage and side vents were covered with Nytex® (250-micron mesh opening; BioQuip Products, Rancho Dominguez, CA). The cage provides a barrier against insects and prevents cross contamination between colonies. Avirulent WCM colonies were kept in separate growth chambers on a 14:10 (L:D) cycle maintained at 27°C. Colony was maintained by transferring fifty mites onto 10 new wheat plants every two-three weeks.

Single Mite Transfers. Source plants were established in the greenhouse using 'Millennium' wheat seeded into 4-cm diameter cone-tainers™ (Stuewe & Sons Inc., Tangent, Oregon, USA) filled with standard greenhouse soil. Cone-tainers were covered with plastic cylindrical cages (5-cm in diameter and 50-cm in height) with two to three vents, covered with Nytex® screen.

Ten days after seeding (two-three leaf stage) half of the plants were inoculated with TriMV and the other half with sterilized distilled water (Mock). For TriMV inoculations, TriMV positive tissue was ground in sterilized distilled water at a 1:20 wt/vol ratio using a mortar and pestle. Plants were dusted with carborundum to induce scarring of plant tissue. Leaves were placed in the palm of one hand, and a pestle was dipped in the solution and applied to the leaves using moderate pressure. Mock inoculations were done in the same manner using only sterilized distilled water.

One week after inoculation, ten WCM were transferred from each of three avirulent wheat curl mite colonies (Nebraska, South Dakota, Montana) onto three TriMV and three mock-inoculated source plants. To transfer mites from colonies to source plants, the mites were placed onto black insect mounting triangles using a human eyelash attached to a wooden dowel. Triangles were placed into the axil of the newly emerging leaf of each source plant. Plants remained in the lab for a minimum of 10 hours following the transfer to allow mites to become established at which time they were transferred to a growth chamber with a 14:10 (L:D) cycle maintained at 27°C. Mites were permitted to build up on source plants for a period of three weeks.

Test plants were seeded one week after infesting source plants with mites. One 'Millennium' wheat seed was seeded per cone-tainer and covered with cages immediately after seeding. When test plants reached the three-leaf stage (14 days after planting), individual mites were transferred from each source plant onto 10 test plants. To transfer mites to test plants, a source plant containing mites was cut at soil level and placed under

a stereo microscope. One mite was removed from the source plant using a human eyelash attached to a wooden dowel and brought to an adjacent stereo microscope with a test plant tilted at a 30° angle with the newly emerging leaf in focus. The mite was placed directly on the inner whorl of the newly emerging leaf and observed until it appeared established. Cone-tainers were then brought to an upright position, covered with cages and the base sealed with tape. Only adults and second instars exhibiting normal movement were transferred. After transferring, test plants remained in the lab for a minimum of 10 hours to allow mites to become established. Source plants were placed individually into plastic zip-lock bags and stored at -20°C for subsequent indirect enzyme-linked immunoabsorbent assay (ELISA). Test plants were transferred to a growth chamber maintained at 27°C.

After three-four weeks test plants were harvested. Test plants were cut at soil level and examined under a stereomicroscope to determine mite presence and virus symptomology. Mite presence was determined based on four classes (0= 0 mites present, 1 = 1 to 10 mites present, 2 = 10 to 100 mites present, 3= >100 mites present). Each test plant was put into individual zip-lock bags and stored at -20°C for ELISA. This procedure was repeated five times with a total of 15 source plants and 150 test plants per treatment combination.

Multi-Mite Transfers. Seifers *et al.* (2008) indicated that WCM exhibited very low transmission rates of TriMV. This study was designed to address the issue associated with low transmission rates. Source plants were established using the same procedure as

the single mite transfers with the exception of three plants/cone-tainer. Thirty-six source plants were inoculated with TriMV and 24 were mock inoculated with sterilized distilled water at the two-three leaf stage (ca. 14 days). Seven days after inoculation, ten avirulent mites were transferred onto each of the three source plants in the cone-tainers. Only adults and second instars exhibiting normal movement were transferred. Each WCM colony (Nebraska, Montana and South Dakota) was transferred onto eight TriMV cone-tainers and six Mock inoculated cone-tainers. Source plants remained in the lab for 10 hours before being transferred to the growth chamber. Mites were permitted to build up on source plants for a period of one week.

Test plants were seeded in 21, 15-cm-diameter pots. Two empty cone-tainers were buried at a normal depth in the center of each pot. Ten 'Millennium' wheat seeds were planted at a depth of one inch around the outside of the cone-tainers in each pot. Pots were covered with 15-cm-diameter plastic cages.

To infest test plants, two mite-virus source cone-tainers were placed directly into the empty cone-tainers within each pot containing 14 day old test plants. At this time plant tissue was harvested from each source plant within each cone-tainer for ELISA testing and one entire plant was taken from each cone-tainer to determine mite populations. After infesting test plants with mites, pots were returned to the growth chamber with a 14:10 (L:D) maintained at 27°C. Mites were given four weeks to move from source plants to test plants. After four weeks, all test plants were harvested; mite counts and virus symptoms were recorded for each test plant. Test plants were placed individually into

plastic zip-lock bags and stored at -20°C for ELISA. This experiment was run only once with 4 replications for TriMV and 3 replications for Mock inoculated checks for each colony.

Duplicate samples were tested for TriMV using ELISA (Seifers et al. 2008). Positive TriMV controls consisted of wheat tissue inoculated with TriMV, and healthy wheat tissue was used as a negative control. Step 1: ELISA plates (96 well flat – Bottom Immuno Plate, Maxisorp, Nunc, Thermo Scientific Inc. Dubuque, IA) were coated with TriMV IgG in carbonate buffer at 1:1000 dilution and stored overnight at 4°C. Each sample was prepared by adding wheat tissue along with general extraction buffer [(100 ml of PBST, 2 g of PVP (40,000 wt) and 0.2 g of ovalbumin (crystallized)] at a 1:10 wt/vol. ratio to a mesh bag (Agdia, Elkhart, IN). The sample was ground within the mesh bag using a tissue homogenizer (Agdia). Step 2: 200 µl of the plant tissue solution was taken from the bag and added to each of two sample wells of the ELISA plate. Step 3: 100µl of TriMV IgG-ALP conjugate and general extraction buffer solution (1:500 dilution) was added per well. Plates were incubated at 37°C for one hour and rinsed three times with PBST buffer (1X concentration, Agdia). Step 4: 100 µl of PNP was added to each well and incubated in the dark at room temperature for 1 hour. Quantative measurements of the reaction were determined using absorbance at 405 nm with a Multiscan FC Spectrophotometer (Thermo Scientific Inc. Dubuque, IA).

Both studies were analyzed using PROC FREQ (version 9.2; SAS Institute 2001) to make pairwise comparisons for differences in transmission occurred between colonies.

An analysis of variance was run on the single mite transfer study to determine if significant differences in mite presence using pairwise comparisons between WCM colonies and inoculations (PROC GLIMMIX version 9.2.2; SAS Institute 2008).

Results

Single Mite Transfer Results. All mock-inoculated source plants (45) and test plants
(450) tested negative for TriMV by ELISA assay, confirming that source mites were
avirulent and that no contamination occurred during the study between source plants or
test plants. All TriMV-inoculated source plants (45) tested positive for TriMV
demonstrating that mites transferred to test plants had been well exposed to TriMV.
ELISA was highly sensitive for TriMV with a test ratio ranging from 19 to 38 times the
control. All TriMV source plants tested negative for WSMV by ELISA assay, verifying
that only TriMV was present in the study. Mite population data showed that all plants
were in excess of hundreds of mites per source plant indicating that adequate mite
numbers built up on all source plants three weeks after infestation.

There were no significant differences in transmission between the separate runs
($\chi2=2.14$, $P>\chi2=0.71$, df=4). Therefore, the data were combined and compared to
determine if there were significant differences between colonies. The virus assays
indicated neither Montana nor South Dakota mites (Type 1) transmitted TriMV (Table
2.1). Nebraska (Type 2) mites transmitted TriMV at a high rate of 41% through the single
mite transfers (Table 2.1).

Mite presence data following single mite transfers indicated a significant
difference between virus treated and mock-inoculated test plants (F=6.70, P=0.0114,
df=1). This difference between treatments was due to a reduced survival of 'Nebraska'
mites when transferred from TriMV-inoculated source plants (60%) when compared with

mock-inoculated source plants (84%) (Table 2.2). Single mite transfers from TriMV-inoculated source plants for Montana mites (Type 1) (79%) and South Dakota mites (Type 1) (75%) (Table 2.2) did not exhibit differences in mite presence when transferred from mock and TriMV source plants.

Multi-Mite Study Results. Mite counts indicated mite presence between 11-153 per plant on all source plants prior to infestation of the test pots. ELISA assays also indicated that all source plants except one South Dakota source plant tested positive for TriMV. Source plant data indicates that every pot contained test plants had adequate mites and TriMV present for virus transmission. In addition, all source plants tested negative for WSMV. After exposure to the source plants for three weeks, the test plants all had mites in excess of thousands per plant. Thus, mites infested the test plants within each pot early in the study, providing extensive exposure of mites for TriMV transmission. All mock source plants and test plants were negative for TriMV indicating no virus contamination of the source mite colonies or movement of mites between pots. Under these extreme conditions, Nebraska mites (Type 2) transmitted TriMV to 100% of the test plants. Montana and South Dakota (Type 1) mites transmitted at a much lower rate of 2.5% (Table 1.2).

Discussion

This study demonstrated that TriMV is differentially transmitted by WCM types. When using single mite transfers, TriMV was only transmitted by 'Nebraska' mites (Type 2). Type 1 (Montana and South Dakota) mites were unable to transmit TriMV except when very high mite numbers were used. Seifers *et al.* (2009) determined that the transmission rate of TriMV by the wheat curl mite was ca. 2%. Although the type of wheat curl mites used by Seifers *et al.* (2009) was not determined, our data would indicate that they were not Type 2 WCM. The results from this study indicate the importance of determining the mite type when evaluating studies where TriMV transmission is important. In addition, studies conducted in a field setting should consider the types of mites present.

Mite presence data indicated that mite survival was significantly lower for mites transferred from TriMV-inoculated source plants. Further studies will need to be conducted to determine the impact of TriMV on the biology of the WCM. Siriwetwiwat (2006) demonstrated that WSMV presence significantly increased the reproductive rate for 'Nebraska' (Type 2) but not Type 1 WCM.

Studies on the transmission of *Wheat mosaic virus* (WMoV) have also demonstrated differential transmission by these same mite types. Seifers *et al.* (2002) showed that Nebraska (Type 2) were the most efficient vector of WMoV. This type of WCM was also identified as the most efficient vector of TriMV. WMoV transmission was significantly higher for 'Montana' mites when source plants were co-infected with

WSMV. The colonies used in this study were derived from the same colonies Seifers *et al.* (2002) used to determine mite transmission of WMoV. Further studies are needed to determine if differences in TriMV transmission with WSMV co-infected plants.

TriMV and WSMV have been shown to have synergistic impacts on infected plants in greenhouse trials, increasing the potential for economic impact in field situations (Tatineni et al. 2010; Byamukama *et al.* 2012). This study indicates that the economic impact of the virus complex may vary relative to the abundance and type of mite present. Siriwetwiwat (2006) in testing the genetic variability of WCM types in the field found that the majority of genetic variability occurred within a wheat head. The study also indicated that there were no significant differences in genetic variability between fields or states sampled. However, there were differences in the relative abundance of the mite types between states. A survey of symptomatic plants from nine states in the Great Plains indicated that TriMV was widespread throughout the region (Burrows *et al.* 2008). The study also indicated that there were large differences in the percentage of plants positive for TriMV. The results from this study could have an important impact in determing the epidemiology of TriMV and its associated viruses.

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Tables

Table 2.1. TriMV transmission by various wheat curl mite colonies for single mite and mult-mite transfer studies.

	No. of Positive TriMV plants / Total TriMV transferred plants (%)	
WCM Source	Single Mite Transfer	Multi-Mite Transfer
Montana	0/150 (0)	1/40 (2.5)
Nebraska	61/150 (41)	40/40 (100)
South Dakota	0/150 (0)	1/40 (2.5)
$\chi 2$	141.1311	111.4286
P> $\chi 2$	<0.0001	<0.0001
Df	2	2

Table 2.2. Mite presence on mock- and TriMV- inoculated test plants between colonies using single mite transfer.

	WCM Infested/Total Plants (%)				
WCM Source	Mock	TriMV	t value	Pr >	d.f
Montana	123/150 (82%)	115/150 (76%)	0.50	0.6176	1
Nebraska	126/150 (84%)	90/150 (60%)	3.29	0.0015	1
South Dakota	120/150 (80%)	114/150 (76%)	0.75	0.4573	1
t value	0.13	3.72			
Pr >	0.8815	0.0327			
df	2	2			

CHAPTER 3

Impact of Triticum mosaic virus on the biology of the Wheat Curl Mite (*Aceria tosichella* Keifer)

Introduction

The wheat curl mite (WCM) *Aceria tosichella* Keifer is the only known vector of three viruses in wheat; *Wheat streak mosaic virus* (WSMV), *Wheat mosaic virus* (WMoV) and *Triticum mosaic virus* (TriMV). These three viruses collectively make up the wheat-mite-virus complex. During the 2011 growing season, this virus complex was the second most important disease with an average loss of 1.7% in the western Great Plains (Appel *et al.* 2011). TriMV is the most recently discovered virus within this wheat-mite-virus complex. TriMV was first identified in Kansas wheat research plots during the 2006 growing season (Seifers *et al.* 2008). Since its discovery, TriMV has been found in Colorado, Montana, Nebraska, Oklahoma, South Dakota, Texas, and Wyoming (Burrows *et al.* 2008). In the field, TriMV is most often found in a co-infection with WSMV. Greenhouse studies indicate that wheat plants co-infected with TriMV and WSMV result in a higher titer for both viruses. This synergistic interaction between these viruses increases their potential impact on wheat yields (Tatineni *et al.* 2010; Byamukama *et al.* 2012).

WCM are vermiform, white in color, and are approximately 150-250 μm in length (Keifer 1938). Due to their small size, WCM are barely visible to the naked eye. The complete lifecycle of a WCM requires 7-10 days (Staples and Allington 1956). WCM reproduce by arrhentokous parthenogenesis with no copulation occurring (Helle and Wysoki 1983). Males deposit spermatophores on the leaf surface and females will pick them up later. Unfertilized females will produce only haploid males, but fertilized

females produce both haploid males and diploid females. Each female is capable of laying approximately 12-20 eggs during her lifetime. Although no studies have been conducted, it is estimated that adults can live approximately 30 days. WCM populations can build rapidly under favorable conditions. It has been estimated that under ideal conditions a single female can result in 3 million mites after 60 days.

WCM movement between plants is passive and assisted by wind currents (Sabelis and Bruin 1996). After landing on a host, mites migrate to the whorl of newly emerging leaves, where they feed and are protected from desiccation and predators (Slykhuis 1955). The extent of movement of WCM between plants is dependent on the density of mite populations, with greater population buildup and thus, greater movement potential occurring from more healthy wheat plants (Thomas and Hein 2003). Mite movement is a critical component to the secondary spread of virus in the field.

To reduce the economic impact of this virus complex, WCM resistant wheat varieties were deployed in the late-1980's (Porter *et al.* 1987). By the mid-1990's, resistant-breaking WCM populations had been identified in Kansas, Nebraska, South Dakota, Texas, and Montana. These populations varied in their response to wheat resistant varieties depending on the location where they had been collected (Harvey *et al.* 1995; Harvey *et al.* 1999). Malik *et al.* (2003) in screening for potential resistance summarized the populations into three 'biotypes' classified as 'Nebraska', 'Kansas', and 'Montana'. The differences in these biotypes have implications for deploying mite resistant varieties of wheat.

Genetic differences between WCM populations were first identified in Australia (Carew *et al.* 2009). In Australia, two mite genotypes were identified through mitochondrial 16S rRNA and two nuclear markers. Hein *et al.* (unpublished) tested the same WCM strains that Harvey *et al.* (1999) tested for mite resistance to wheat varieties. Genetic differences were determined using mitochondrial and ribosomal DNA. The results indicated that North American populations consisted of two distinct genotypes that corresponded to the Type 1 and Type 2 mites identified in Australia. The 'Nebraska' (Type 2) population was genetically distinct from the 'Kansas', 'Montana', 'South Dakota', and 'Texas' (all Type 1) populations. The scale of genetic differences between these two types was comparable to their differences with *Aceria tulipae* Keifer, indicating the extent of diversity between these WCM types.

Several studies have shown the complexity of plant-pathogen-vector interactions (Jensen et al. 1969). Interactions between these organisms can be direct or indirect and range from highly beneficial to lethal. Plant viruses have been shown to alter the rate of increase, reproductive period, attraction, behavior, morphology, longevity, and fecundity of their vectors (Fereres et al. 1989; Miller and Coon 1964). Alterations in the biology and ecology of vectors can have a significant impact on the distribution and frequency of a virus in the environment. Siriwetwiwat (2006) determined that WSMV significantly increased the reproduction rates of Type 2 ('Nebraska') mites by up to three times. These increasing rates of reproduction could lead to the development of higher mite populations

earlier in the growing season, and mite movement has been shown to be positively correlated with mite populations on plants (Thomas and Hein 2003).

In a study conducted to determine the transmission of TriMV using wheat curl mites collected across the Great Plains regions indicated that Type 2 (Nebraska) mites transferred from TriMV inoculated plants showed a significant reduction in survival (see Chapter 2). Therefore, further research is needed to determine the impact of TriMV on mite reproduction. The objective of this study was to determine the impact of TriMV on the reproductive rate of wheat curl mite populations collected in the Great Plains region.

Materials and Methods

Three WCM populations were used in this study designated as 'Nebraska' (Type 2), and two populations of Type 1, 'Montana' and 'South Dakota'. These were the same populations Harvey *et al.* (1999) used to evaluate mite resistance to WCM resistant wheat varieties, Seifers *et al.* 2002 used to evaluate the transmission of WMoV, and Hein (unpublished) used to characterize the mites by PCR-RFLP. These WCM populations were maintained on 'Millennium' wheat and isolated by using cages and physical separation. Cages were made of a 15-cm-diameter plastic with two 8-cm-diameter ventilation holes on opposite sides and the top covered with Nytex® (225 x 326 mesh). Avirulent WCM colonies were kept in separate growth chambers with a 14:10 (L:D) cycle maintained at 27°C, and 50 mites were transferred onto new plants every two to three weeks.

10-Mite Transfer Method. 'Millennium' wheat plants were established in 15-cm-diameter pots and pots were caged after seeding. Ten days after seeding, half of the pots were inoculated with TriMV (nine pots) and the other half were inoculated with distilled water (mock). For TriMV inoculations, TriMV-positive tissue was ground in sterilized distilled water at a 1:20 wt/vol ratio using a mortar and pestle. To inoculate, plants were dusted with carborundum to induce scarring of plant tissue. Leaves were placed in the palm of one hand while a pestle was dipped in the solution and applied to the leaves using moderate pressure. Mock inoculations were done in the same manner using only sterilized distilled water. Five days after inoculation each wheat plant within a pot was

infested with 10 WCM. WCM from each of the three populations were transferred onto three TriMV-inoculated pots and three mock-inoculated pots. To transfer mites from colonies to test plants, wheat tissue from each source was inspected under a stereo microscope at 30-40X and ten avirulent mites were placed onto a black insect mounting triangle by using a transfer tool made from a human eyelash attached to a wooden dowel. The triangle was then placed in the axil of each of the four test plants within a pot. Only adults and second instars exhibiting normal movement were transferred. After infestation, pots remained in the lab for a period of 10 hours to enable mites to settle on the plants. Plants were then transferred to a growth chamber with 14:10 (L:D) cycle maintained at 27°C.

One test plant was randomly harvested from each pot at 7, 14 and 21 days after infestation. Sampled plants were cut at soil level, placed in zip-lock bags and stored at 4°C until mites were counted. All mites and eggs on plants were counted using a stereo microscope (magnification ca. 30-40X). After counting, plants were stored at -20°C for later virus assay. The experiment was conducted four times with each run consisting of three replications for each treatment with a total of 12 replications for each treatment.

Single Mite Transfer Method. 'Millennium' wheat plants were established in 4-cm diameter cone-tainers (Stuewe & Sons, Inc., Tangent, OR) that were covered with plastic cylindrical cages after seeding. Cages consisted of 4-cm diameter plastic tubing with two to three vents covered with Nytex® screen (225 x 326 mesh) to reduce the movement of mites between treatments. Ten days after seeding (two to three leaf stage) half of the

plants were inoculated with TriMV and the other half with sterilized distilled water (mock) as per the procedures described above. Five days after inoculation, individual mites from each colony were transferred onto 28 TriMV and 28 Mock inoculated plants as described above. Test plants remained in the lab for minimum of 10 hours and were then transferred to a growth chamber with 14:10 (L:D) cycle maintained at 27°C

Seven randomly selected plants were collected 7, 14, 21, and 28 days after infestation. Plants were cut at soil level and stored at 4°C until mites were counted. Of the seven plants collected, only the first three plants containing mites were counted. Occasional presence of thrips occurred in the test plants, but only plants with established mite populations and without visible thrips damage were counted. All mites and eggs were counted on test plants. Total plant height was recorded as well as the highest point at which WCM were found on the plants. Plants were stored in zip-lock bags at -20°C for later virus assay. Each run consisted of three replications and the experiment was conducted three times with three replications per run.

Virus Assay

Duplicate samples were tested using an indirect enzyme-linked immunoabsorbent assay (ELISA) for TriMV. TriMV-infected wheat tissue was used as a positive control and healthy wheat tissue was used as a negative control. ELISA plates (Immuno Plate, Maxisorp, Thermo-Fisher Scientific, Waltham, MA) were coated with TriMV IgG in carbonate buffer at a 1:1000 dilution and stored at 4°C overnight. Samples were prepared

by adding wheat tissue with general extraction buffer (100 ml PBST, two grams 40,000-wt PVP and 0.2 grams of ovalbumin) at 1:10 wt/vol. ratio to a mesh sample bag (Agdia, Elkhart, IN). Samples were ground by using a tissue homogenizer (Agdia). Plant tissue liquid (200 µl) was extracted from the mesh bag and added to each sample well of the ELISA plate with duplicate wells per sample. Next, 100µl of TriMV IgG-ALP (alkaline phosphatase) conjugate in general extraction buffer solution (1:500 dilution of conjugate:buffer) were added to each well. Plates were incubated at 37°C for one hour and rinsed three times with PBST buffer (1X concentrate, Agdia). Finally, 100 µl of PNP (Agdia) were added to each well and incubated in the dark for one hour. Sample absorbance (405 nm) was measured with a Multiscan FC plate reader (Thermo-Fisher Scientific). Effectiveness of each ELISA run was always verified by using positive and negative controls on each plate.

PROC GLIMMIX (version 9.22; SAS Institute 2008) with repeated measures design was used to analyze the 10-mite transfer data to compare treatments. PROC GLIMMIX without repeated measures was run on the single mite transfer experiment. Single mite transfer was analyzed without repeated measure because experimental units were not subject to the same conditions because each plant was a separate experimental unit.

An initial analysis using studentized residuals indicated that the data were not normally distributed. Variances increased geometrically as a function of the mean indicating a negative binomial distribution. Due to the negative binomial distribution, the

subsequent estimations are most appropriate for a mixed model method (Gbur *et al.* 2012). Data were transformed to natural log prior to analysis. An analysis of variance was run to determine the significance of main effects and interactions. These effects were partitioned over day into linear and quadratic portions to determine which fixed effects were significant. Non-significant effects were removed from the model. The analysis of variance was run again containing only the significant effects. Regression equations were obtained from the solution for fixed effects and slope comparisons were made between treatments. In a generalized linear mixed model, R^2s are understood as undefined. However, the correlation between observed values and the values predicted by the regression equations resulting from the analysis above can be used to estimate the fit of the equations. Correlations were obtained through PROC CORR (version 9.22; SAS Institute 2008).

Results

10-Mite Transfers Method. All mock-inoculated (108) wheat plants tested negative for TriMV and all TriMV-inoculated (108) plants tested positive for TriMV. None of the TriMV-inoculated source plants were positive for WSMV. Plants positive for TriMV ranged from 19 to 31 times the control based on A_{405} (Absorbance at 405-nm wavelength). Plant health ratings taken at plant harvest indicated that, as yet, there were no significant impacts on plant health between inoculations (mock vs. TriMV). However, mild symptoms were visible on TriMV-inoculated plants, but the wheat plants showed no significant chlorosis.

The analysis of variance type I test for fixed effects (Table 3.1; 3.2) indicated that there were no significant differences between colonies for mites or eggs. Significant differences occurred between inoculations (mock- vs. TriMV-inoculated plants) for both mites and eggs. Differences across sampling days were also significant for both mites and eggs indicating a significant change in mite population over the sampling period. There was a significant day by colony interaction for mites but not for eggs, indicating that colonies changed relative to one another over time. Significant day by inoculation interaction occurred for both mite and eggs (Table 2.1, 2.2) signifying changes between inoculations over time. Day by day interaction was also significant for both mites and eggs indicating the presence of a quadratic relationship. There were no significant interactions between the quadratic and the treatments (colony or inoculation).

Table 3.3 and 3.4 show the quadratic equations of mites and eggs for WCM colonies. Each of these predicted equations are represented in graphs with the observed values in Figures 3.1-3.3. Correlations between predicted and observed values for these equations ranged from 0.77-0.92 for mites and 0.72-0.84 for eggs, indicating that prediction equations were a good representation for observed values.

Equations for each colony contain different intercepts for each inoculation due to the significant main effect of inoculation differences. The linear portion of each equation is unique to each colony and inoculation combination due to differences in both inoculation and colony over time. The quadratic portion had no significant interaction with treatments (colony or inoculation); therefore the quadratic (day*day) term is the same for all equations. There were no significant differences between colonies; however, there were significant differences between inoculations with each colony (Table 3.1; 3.2). Inoculation differences resulted in a reduction in population build up for both mites and eggs on all WCM colonies when reared on TriMV-inoculated plants compared to mites reared on mock-inoculated plants.

Single Mite Transfer Method. All mock-inoculated (108) plants tested negative for TriMV and all TriMV-inoculated (108) except four were positive for TriMV. Plants that were not positive for TriMV were not used in the analysis. None of the TriMV-inoculated source plants tested positive for WSMV. TriMV positive plants ranged from 18 to 28 times the control.

The analysis of variance type I test for fixed effects (Table 3.5; 3.6) indicated a significant inoculation effect for both mites and eggs. The main effect of colony was only significant for mites. There was significant colony by inoculation interaction for both mites and eggs. The day by colony interaction was approaching significance for mites and eggs. Day by inoculation interactions was only significant for mites.

Table 3.7 and 3.8 represent the quadratic equations for mites and eggs using single mite transfers, respectively. Mites and eggs have different intercepts for each inoculation combination due to the significant colony by inoculation interaction that occurred for both response variables. For mites, the linear portion of the equation is different for each equation due to significant day by colony and day by inoculation interactions. Linear portions of the equation for eggs approached significance for day by colony and were therefore included in the model. The inoculation by day interactions for eggs was not significant and was removed from the model. Figure 3.4 to 3.6 show graphs of the predicted equations for mites and eggs as well as the observed data. Correlations between observed and predicted values range from 0.93-0.97 for mites and 0.63-0.94 for eggs indicating that prediction equations were a good fit for the observed values.

Least squares means differences for the single mite transfers showed significantly lower mite populations on TriMV- inoculated plants compared to mock-inoculated for South Dakota (Type 1) and Nebraska (Type 2) mites. Montana (MT) Type 1 mites were not significantly different between TriMV- and mock- inoculated plants. Egg populations

were significantly lower on TriMV-inoculated plants for all colonies when compared with mock-inoculated plants using single mite transfers.

Discussion

The 10-mite transfer and single mite transfer studies confirmed that TriMV had a significant negative impact on mite populations. The main effect of inoculation (TriMV- vs. mock-inoculated plants) was highly significant for mites and eggs in both studies. Colony differences were significant only for mites when single mite transfers were used.

The result obtained from the single mite transfer study was representative of what was found for the 10-mite transfer method. Both of these studies indicated a significant inoculation effect, lowering the reproductive potential for WCM when reared on TriMV-infected plants compared to mock-inoculated controls. However, Montana (Type 1) mite were not significantly affected by TriMV using the single mite transfer method. Although Montana (Type 1) mites were not significantly impacted in that study there was a numerical decrease in the population over time for TriMV-inoculated plants compared to mock-inoculated plants.

Siriwetwiwat (2006) compared the reproductive rate of WCM feeding on WSMV-inoculated compared to mock-inoculated plants for multiple mite colonies and found an increased reproductive rate for Type 2 (Nebraska) mites only. The study also demonstrated that this effect was not observed with other viruses infecting wheat that are not transmitted by the WCM. Siriwetwiwat proposed the increase in Nebraska (Type 2) mites was due to a longer evolutionary history with WSMV than Montana and South Dakota (Type 1) mites.

WSMV was first identified in 1922 as 'yellow mosaic' (Staples and Allington 1956). It is widespread through the Great Plains with multiple isolates. The genetic diversity of WSMV indicates that it has been present in the Great Plains longer than TriMV which was only recently discovered in 2006 (Seifers *et al.* 2008). The limited coevolution of TriMV and WCM could be a partial explanation for the negative impact on mite reproduction.

There are multiple other factors that could be responsible for the negative impact of TriMV on WCM populations. TriMV-inoculated plants may have a lower nutritional quality or cause an increase in the production of secondary metabolites that are disadvantageous to the WCM. Tatineni (*unpublished*) observed toxicity of injectious cDNA clones of TriMV to *Escherichia coli* strain JM109 (Migula) might suggest that some of TriMV proteins might be toxic to WCM as well.

Plant viruses have been shown to alter the performance of their vectors in other systems (Mowry 1994; Fiebig *et al.* 2004; Eubanks *et al.* 2005). The magnitude of these interactions and sign of the interaction varied depending on the taxa involved (Donaldson and Gratton 2007). Blua and Perring (1992) showed that *Zucchini yellow mosaic virus* had a significant negative impact on the survival and fecundity of the *Aphis gossypii* Glover. *Soybean mosaic virus*, a virus within the same family as TriMV significantly reduced soybean aphid (*Aphis glycines* Matsumura) populations by 50% in field conditions, and by 25% in laboratory conditions (Donaldson and Gratton 2007).

Our study is the first report of a virus within this wheat-mite-virus complex to show a negative impact on its vector. The reduction of WCM populations reared on TriMV-inoculated wheat plants may have an impact on the secondary spread of the virus in field situations. A survey of the symptomatic plants in the Great Plains region indicated a higher presence of WSMV (47%) than TriMV (17%) (Burrows *et al.* 2008). Our study provides some evidence for the reduction in TriMV presence may be due to the reduced reproductive rate of WCM on TriMV infected plants. This is supported by evidence provided by Thomas and Hein (2003) who determined that increasing mite densities were strongly correlated with mite movement and thus virus spread. However, field studies on secondary spread of WCM with TriMV will need to be conducted to determine if the results from this experiment have an impact on virus spread under field conditions.

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Tables and Charts

Table 3.1. Analysis of variance type I test for fixed effects on mites for colony, inoculation and day using 10-mite transfers (Colony = MT, SD, and NE, Inoculation = Mock and TriMV, Day = 7, 14, 21).

Effect	Num DF	Den DF	F-Value	Pr > F
Colony	2	180	0.12	0.8832
Inoculation	1	180	84.93	<.0001
Colony*Inoculation	2	180	0.82	0.4406
Day	1	180	2254.26	<.0001
Day*Colony	2	180	5.17	0.0066
Day*Inoculation	1	180	13.06	0.0004
Day*Colony*Inoculation	2	180	0.80	0.4505
Day*Day	1	180	30.13	<.0001
Day*Day*Colony	2	180	0.35	0.7054
Day*Day*Inoculation	1	180	1.06	0.3041
Day*Day*Colony*Inoculation	2	180	1.02	0.3633

Table 3.2. Analysis of variance type I test for fixed effects on eggs for colony, inoculation and day using 10-mite transfer method (Colony = MT, SD, and NE, Inoculation = Mock and TriMV, Day = 7, 14, 21).

Effect	Num DF	Den DF	F-Value	Pr > F
Colony	2	179	0.02	0.9849
Inoculation	1	179	28.17	<.0001
Colony*Inoculation	2	179	0.15	0.8612
Day	1	179	1589.95	<.0001
Day*Colony	2	179	1.93	0.1487
Day*Inoculation	1	179	6.71	0.0104
Day*Colony*Inoculation	2	179	1.38	0.2532
Day*Day	1	179	83.80	<.0001
Day*Day*Colony	2	179	0.06	0.9413
Day*Day*Inoculation	1	179	0.50	0.4824
Day*Day*Colony*Inoculation	2	179	0.09	0.9103

Table 3.3. Regression equations after natural log-transformation for WCM mite buildup for each of the WCM colonies on mock- and TriMV-infected wheat plants using 10-mite transfer method.

WCM Colony	Inoculation	Equation	Correlation	n
Montana	Mock	$Y=1.7585+0.4509x-0.00675x^2$	0.84510	36
	TriMV	$Y=1.7486+0.4050x-0.00675x^2$	0.86176	36
Nebraska	Mock	$Y=1.7585+0.4461x-0.00675x^2$	0.91593	36
	TriMV	$Y=1.7486+0.4138x-0.00675x^2$	0.93943	36
South Dakota	Mock	$Y=1.7585+0.4427x-0.00675x^2$	0.77822	36
	TriMV	$Y=1.7486+0.4096x-0.00675x^2$	0.91309	36

Table 3.4. Regression equations after natural log-transformation for WCM egg buildup for each of the WCM colonies on mock- and TriMV-infected wheat plants using 10-mite transfer method.

WCM Colony	Inoculation	Equation	Correlation	n
Montana	Mock	$Y=-0.4761+0.7178x-0.01550x^2$	0.72321	36
	TriMV	$Y=-0.7011+0.6660x-0.01550x^2$	0.75878	36
Nebraska	Mock	$Y=-0.4761+0.7079x-0.01550x^2$	0.80578	36
	TriMV	$Y=-0.7011+0.6718x-0.01550x^2$	0.84796	36
South Dakota	Mock	$Y=-0.4761+0.7018x-0.01550x^2$	0.66808	36
	TriMV	$Y=-0.7011+0.6671x-0.01550x^2$	0.78431	36

Table 3.5. Analysis of variance type I test for fixed effects on mites for colony, inoculation and day using single mite transfer method (Colony = MT, SD, and NE, Inoculation = mock and TriMV, Day = 7, 14, 21).

Effect	Num DF	Den DF	F-Value	Pr > F
Colony	2	176	5.49	0.0049
Inoculation	1	176	20.33	<.0001
Colony*Inoculation	2	176	3.67	0.0273
Day	1	176	7027.70	<.0001
Day*Colony	2	176	2.52	0.0836
Day*Inoculation	1	176	25.87	0.0001
Day*Colony*Inoculation	2	176	0.37	0.6902
Day*Day	1	176	65.26	<.0001
Day*Day*Colony	2	176	0.82	0.4412
Day*Day*Inoculation	1	176	0.27	0.6018
Day*Day*Colony*Inoculation	2	176	0.05	0.9556

Table 3.6. Analysis of variance type I test for fixed effects on eggs for colony, inoculation and day using single mite transfer method (Colony = MT, SD, and NE, Inoculation = mock and TriMV, Day = 7, 14, 21).

Effect	Num DF	Den DF	F-Value	Pr > F
Colony	2	176	1.34	0.2648
Inoculation	1	176	106.14	<.0001
Colony*Inoculation	2	176	3.57	0.0303
Day	1	176	2694.39	<.0001
Day*Colony	2	176	2.68	0.0712
Day*Inoculation	1	176	1.90	0.1702
Day*Colony*Inoculation	2	176	1.06	0.3485
Day*Day	1	176	106.07	<.0001
Day*Day*Colony	2	176	0.30	0.7382
Day*Day*Inoculation	1	176	0.24	0.6219
Day*Day*Colony*Inoculation	2	176	0.04	0.9623

Table 3.7. Regression equations after natural log-transformation for WCM egg buildup for each of the WCM colonies on mock- and TriMV-infected wheat plants using single mite transfer method.

	Inoculation	Equation	Correlation	n
Montana	Mock	$Y=0.1300+0.4269x-0.00385x^2$	0.94297	36
	TriMV	$Y=0.3254+0.4024x-0.00385x^2$	0.93314	34
Nebraska	Mock	$Y=0.2470+0.4212x-0.00385x^2$	0.94558	36
	TriMV	$Y=0.0163+0.3822x-0.00385x^2$	0.93922	36
South Dakota	Mock	$Y=-0.0146+0.4139x-0.00385x^2$	0.94225	36
	TriMV	$Y=0.0054+0.3838x-0.00385x^2$	0.96557	34

Table 3.8. Regression equations after natural log-transformation for WCM egg buildup for each of the WCM colonies on mock- and TriMV-infected wheat plants using single mite transfer method.

WCM Colony	Inoculation	Equation	Correlation	n
Montana	Mock	$Y=-3.0940+0.6375x-0.00880x^2$	0.80270	36
	TriMV	$Y=-3.8979+0.6375x-0.00880x^2$	0.63410	34
Nebraska	Mock	$Y=-2.5414+0.6150x-0.00880x^2$	0.91485	36
	TriMV	$Y=-3.7074+0.6150x-0.00880x^2$	0.94378	36
South Dakota	Mock	$Y=-2.7044+0.6050x-0.00880x^2$	0.86143	36
	TriMV	$Y=-3.6415+0.6050x-0.00880x^2$	0.87574	34

Table 3.9. Least squares mean differences between 'WCM colonies', reared on mock- and TriMV-inoculated for mites using single mite transfer method (*P-values (P>|t|) for least squares means of WCM significantly different at P<0.05; Standard Error = 0.1820)

Treatment		MT		NE		SD	
	Test Host	Mock	TriMV	Mock	TriMV	Mock	TriMV
MT	Mock	-	-	-	-	-	-
	TriMV	0.2499	-	-	-	-	-
NE	Mock	0.8855	0.3091	-	-	-	-
	TriMV	<.0001*	<.0001*	<.0001*	-	-	-
SD	Mock	0.0343*	0.3543	0.0468	0.0023*	-	-
	TriMV	<.0001*	0.0008*	<.0001*	0.6562	0.0108*	-

Figure 3.1: Relationship of [MT] WCM colony on mock- and TriMV-inoculated plants after natural log transformation using 10-mite transfer method. Top graph represents mite build up, bottom graph represents egg build up.
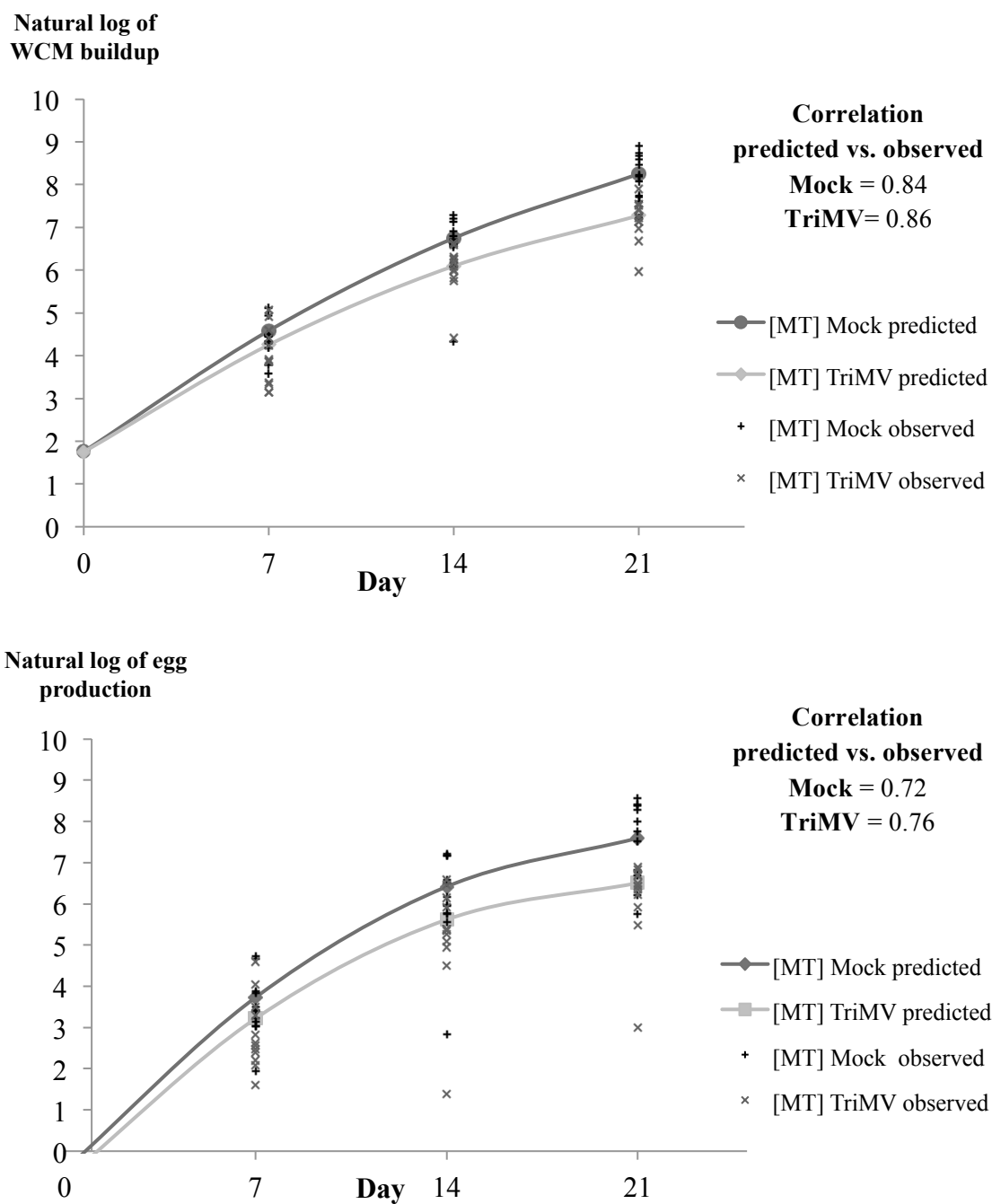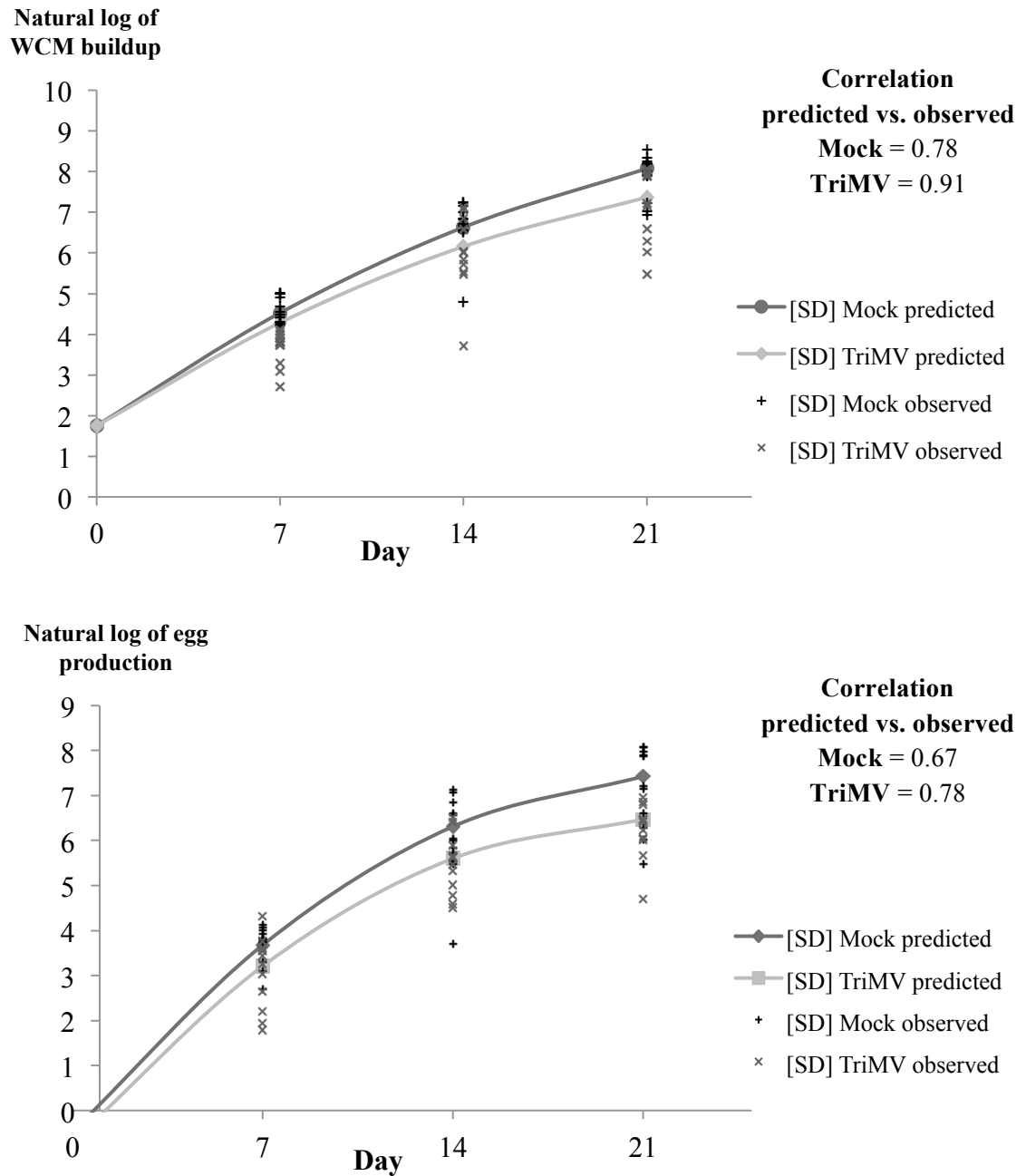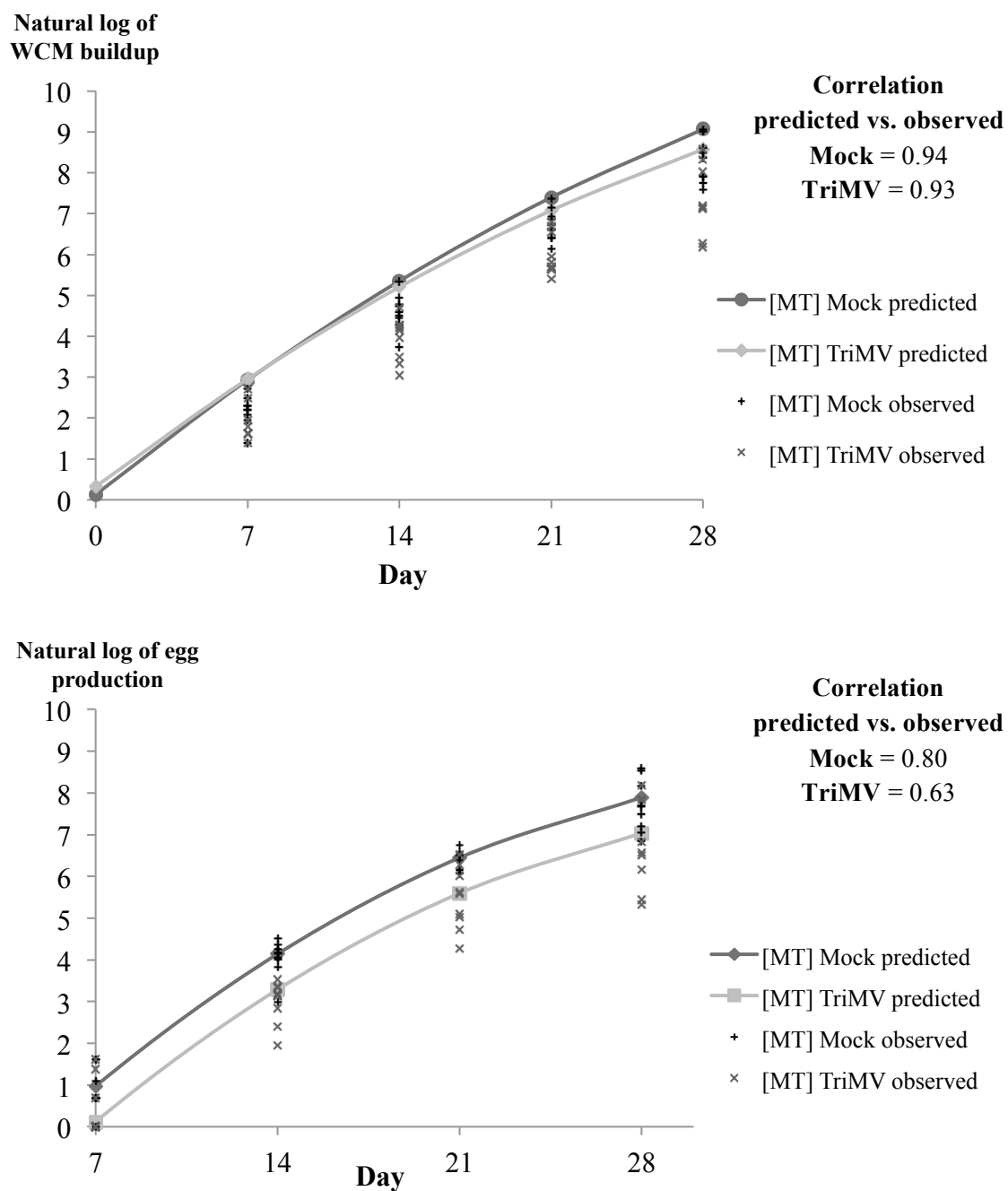
Figure 3.2: Relationship of [NE] WCM colony on mock- and TriMV-inoculated plants after natural log transformation using 10-mite transfer method. Top graph represents mite buildup, and bottom graph represents egg buildup.
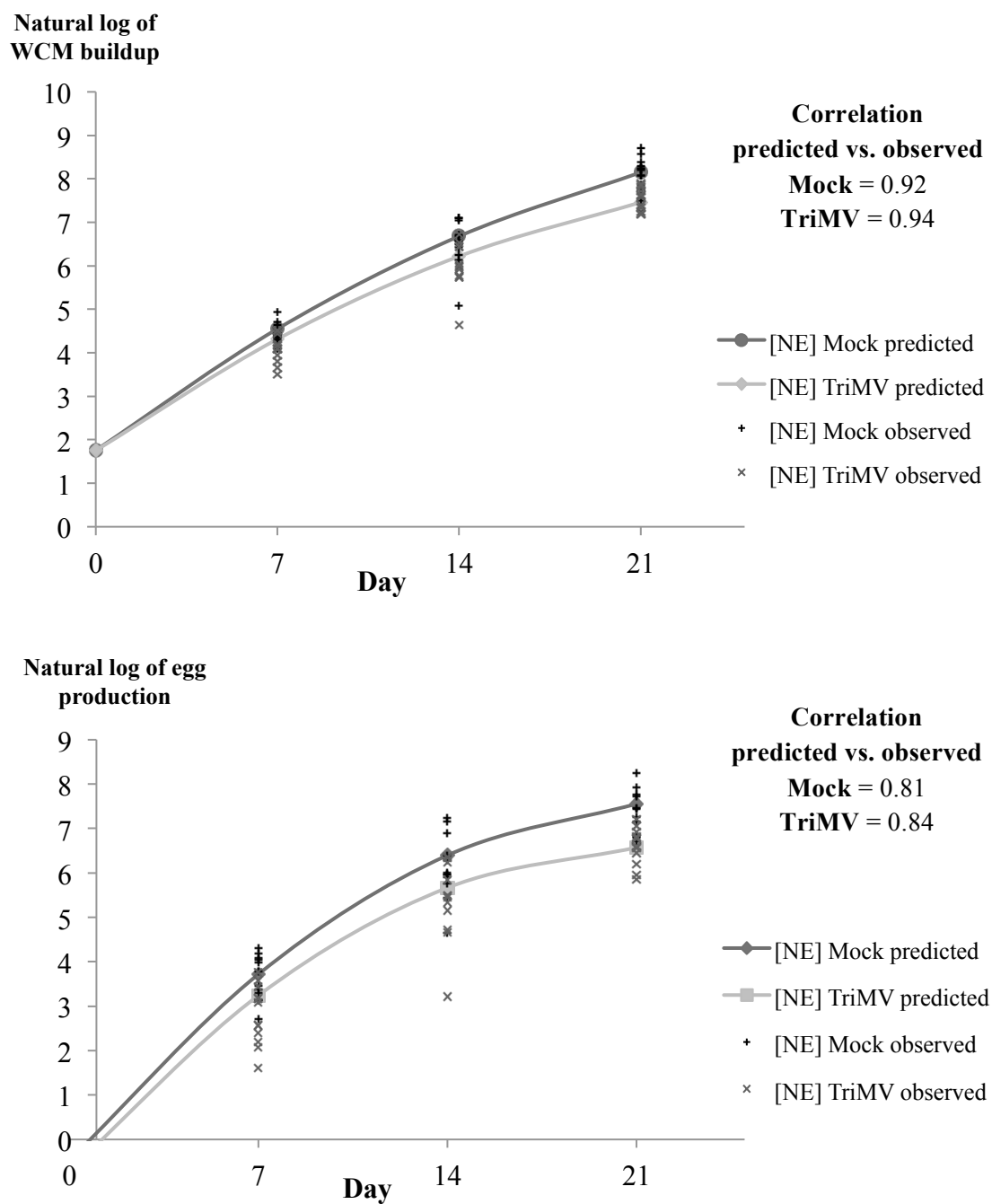
Figure 3.3: Relationship of [SD] WCM colony on mock- and TriMV-inoculated plants after natural log transformation using 10-mite transfer method. Top graph represents mite build up, and bottom graph represents egg build up.

Figure 3.4: Relationship of [MT] WCM colony on mock- and TriMV-inoculated plants after natural log transformation using single mite transfer method. Top graph represents mite build up, bottom graph represents egg build up.
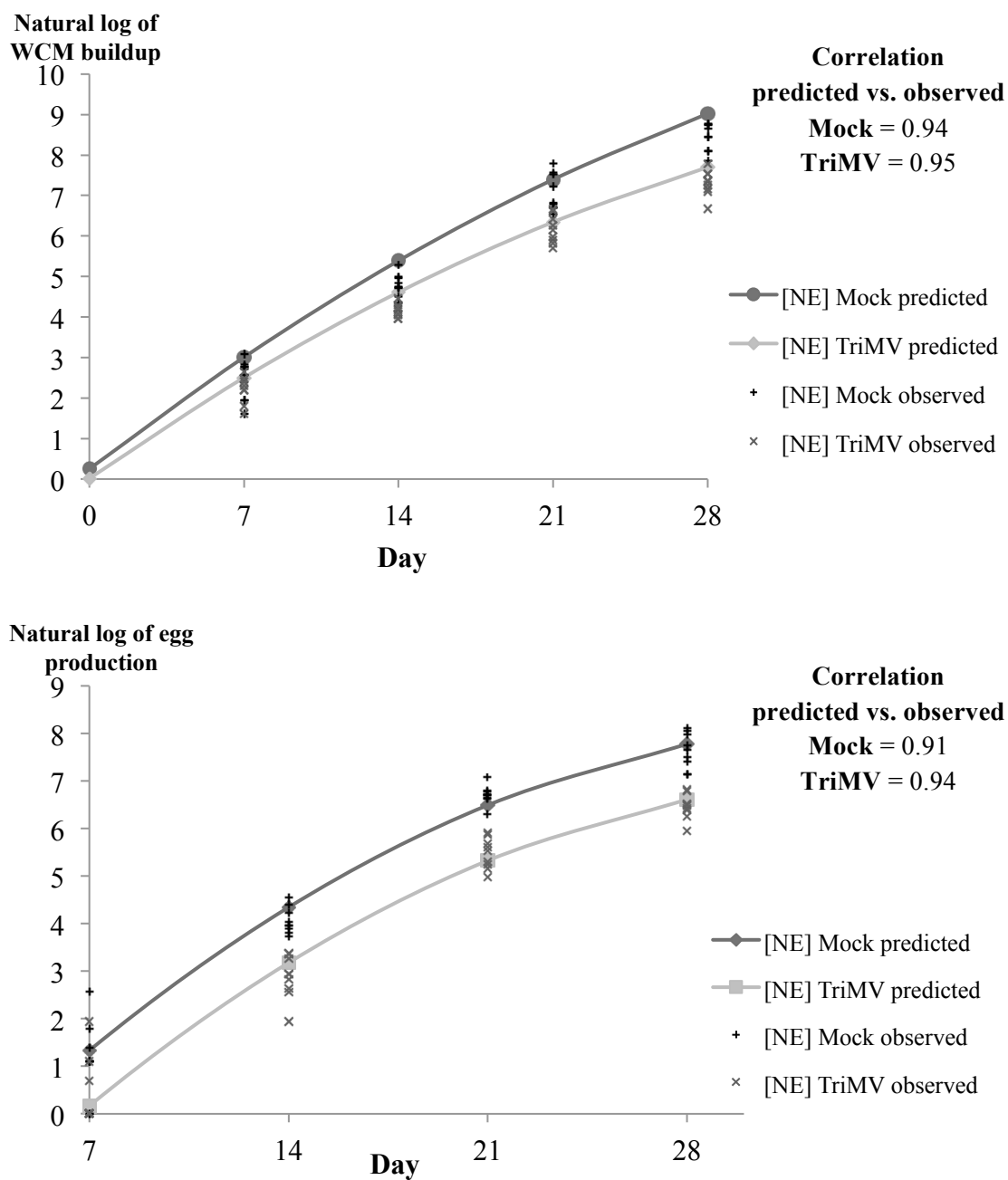
Figure 3.5 Relationship of [NE] WCM colony on mock- and TriMV-inoculated plants after natural log transformation using single mite transfer method. Top graph represents mite build up, bottom graph represents egg build up.

Figure 3.6. Relationship of [SD] WCM colony on mock- and TriMV-inoculated plants after natural log transformation using single mite transfer method. Top graph represents mite build up, bottom graph represents egg build up.
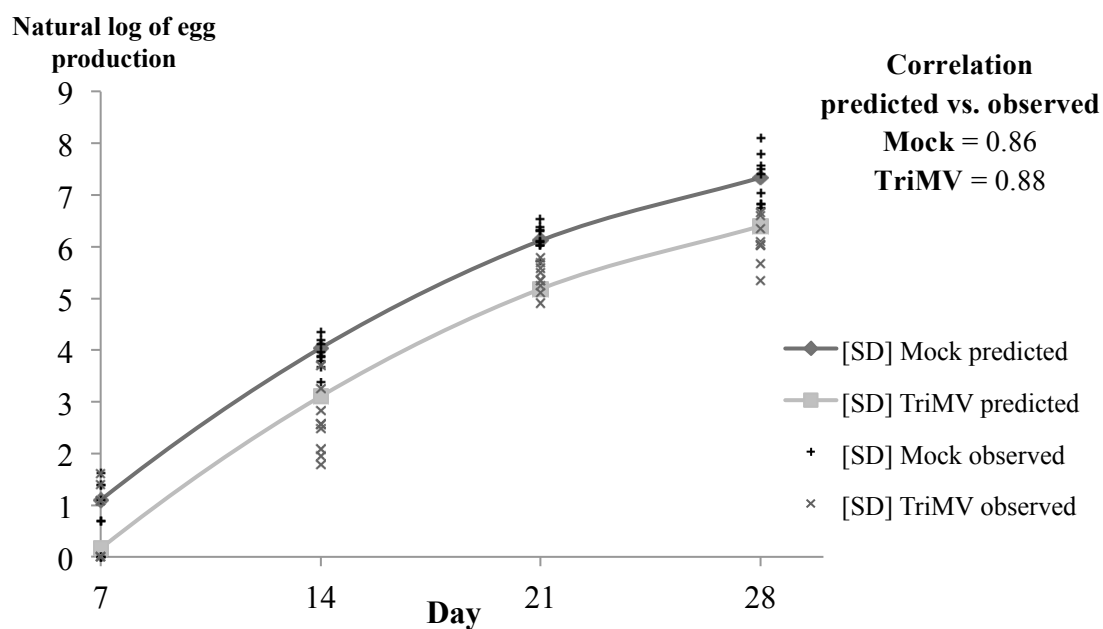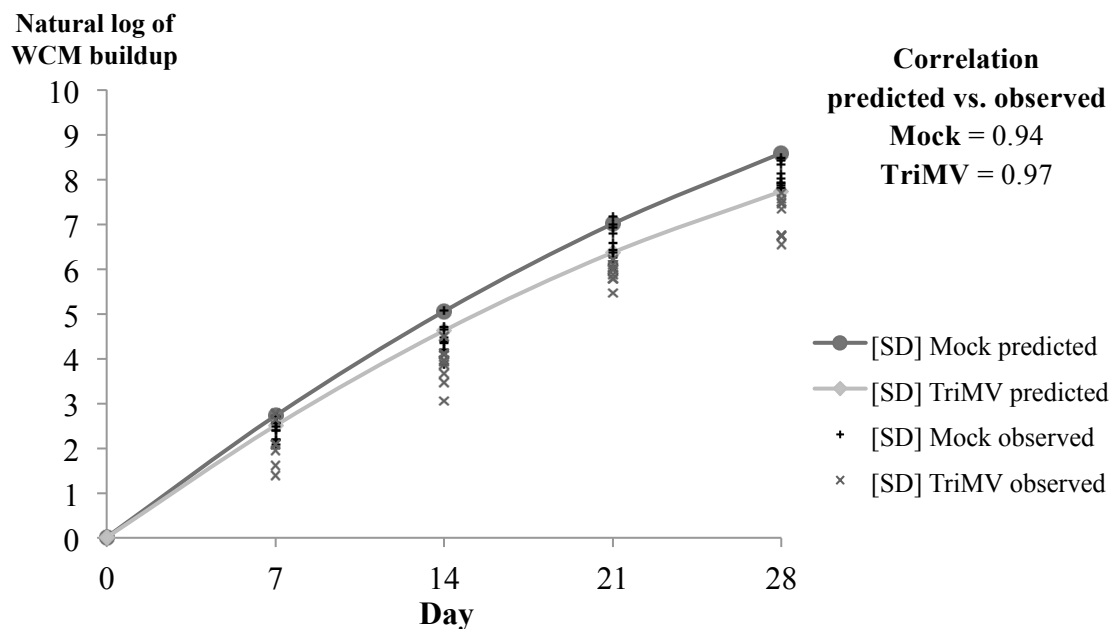
CHAPTER 4

Planting date and variety selection for management of viruses transmitted by the

Wheat Curl Mite (*Aceria tosichella* Keifer)

Introduction

Wheat is an important food grain worldwide and it is the primary dryland crop in the western Great Plains. The wheat-mite-virus complex (*wheat streak mosaic* (WSMV)*, wheat mosaic* (WMoV)*, and Triticum mosaic viruses* (TriMV) is the second largest cause of loss in winter wheat production in Kansas over a 20-year period (Appel et al. 2007). All of the viruses within this complex are transmitted by the wheat curl mite, *Aceria tosichella* Keifer (WCM). Once these viruses become established there are no curative actions; therefore prevention is the key to successful management.

The impact of the wheat-mite-virus complex depends on the plant stage at time of infection, the density of mite populations (infestation level), and temperature during and after infestation and inoculation. Wheat plants infested with mites and infected with virus prior to significant tillering can become stunted, discolored and rosetted (Wegulo *et al.* 2008, Hunger *et al.* 1992; Willis 1984). Infections occurring after wheat is well tillered are not as severe. Density is also critical in determining impact; Thomas and Hein (2003) found that high mite densities were correlated with significant mite movement, resulting in increased spread of the viruses. Temperature is critical to virus replication. Sill and Fellows (1953) found that symptomatic expression of WSMV-inoculated plants held at 28°C occurred in 5 days whereas plants held at 15°C required 15 days. The combination of early virus infection, high mite density and warm weather will maximize the potential virus impact on wheat yields.

Controlling volunteer wheat is considered to be the most effective management tactic for reducing the impact of the wheat-mite-virus complex. However, volunteer wheat control will not always be maximally effective and other important risk factors have been identified. During the 1988 growing season a high incidence of WSMV was reported in eastern Kansas with a low incidence of volunteer wheat (Christian and Willis 1983). Because of the potential loss associated with the wheat-mite-virus complex, producers should consider multiple management tactics to reduce the impact of this complex.

Limited host plant resistance has been developed against viruses transmitted by the WCM. Wheat varieties have also been developed with resistance to the WCM, but mite resistant strains have developed and compromised their effectiveness (Harvey *et al.* 1995, Harvey *et al.* 1999). WSMV resistance has been identified and transferred into wheat (Friebe *et al.* 1991; Gill *et al.* 1995; Wells *et al.* 1973; Well *et al.* 1982). 'Mace' is the first commercial variety released with resistance conferred by the *wsm1* gene (Graybosch *et al.* 2007). Mace has been shown to be effective in reducing the impact of TriMV although its intended release was for the control of WSMV (Tatineni *et al.* 2010, Byamukama *et al.* 2012).

Planting date has also been shown to have a significant effect on the level of severity of WSMV (Willis 1984). It has been observed by several researchers that early planted wheat had a higher severity of WSMV, whereas late seeded wheat reduced the impact of WSMV (Willis 1984; Hansing *et al.* 1950). Early seeding of winter wheat

increases the potential for wheat curl mite establishment and virus infection. Early virus infection can lead to greater virus replication and impact on wheat yields.

Hunger *et al.* (1992) found that early and late planted winter wheat could be significantly impacted by mechanical inoculation of WSMV. Late-planted wheat inoculated in the spring was significantly impacted because of its limited growth. The results of the study indicated that the maturity of plants at time of infection may affect the impact of WSMV.

While previous work has identified the impacts of planting dates and varietal resistance separately, these tactics have not been evaluated in combination. The objective of the current study was to evaluate the combination of planting dates and the resistant variety Mace for their potential at reducing virus impact under high disease pressure. Unlike previous studies conducted on the effects of planting dates and virus impact, this study was conducted using natural populations of wheat curl mites.

Materials and Methods

Field studies were conducted during two separate growing seasons at each of two locations. The 2007-08 and 2008-09 seasons were conducted at the Panhandle Research and Extension Center near Scottsbluff, NE. The 2009-10 and 2010-11 seasons were conducted at the Agricultural Research and Development Center near Mead, NE.

Treatments were arranged in a randomized complete block, split-plot design with four replications. The main plot treatments were planting dates, and the split plot treatments were three winter wheat cultivars. Cultivars were chosen for the study based on their level of resistance to WSMV; Mace (resistant), 'Millennium' (mildly tolerant), and 'Tomahawk' (susceptible). These varieties were planted on three different dates during the fall to simulate early (PD1), recommended (PD2), and late planting (PD3) dates. Each plot consisted of 4, 2-m rows with 0.3-m spacing between rows. In 2007, plots were seeded on 30 August, 21 September, and 9 October. In 2008, plots were seeded on 27 August, 11 September, and 25 September. In 2009, plots were seeded on 25 August, 10 September, and 4 October. In 2010, plots were seeded on 27 August, 15 September, and 5 October.

In the summers of 2007 and 2008, simulated volunteer winter wheat (Millennium) border (ca. 8-m wide) was planted after wheat harvest in late July. Mites were infested into these plots by mid-August by collecting pre-harvest volunteer wheat that was heavily infested with mites and spreading the collected volunteer out over the simulated volunteer

to allow mites to disperse to the growing simulated volunteer. Infested volunteer was located in Kimball County, NE (2007) and Sheridan County, NE (2008).

Prior to the 2009-10 and 2010-11 seasons, simulated volunteer winter wheat border was planted around the plots in May and again in mid-July. Each planting consisted of a 5-m section surrounding the plots with the second planting seeded adjacent to the plots. The volunteer during the summer of 2009 was naturally infested with mites at low levels and these mite populations increased through the summer into the fall. The May planting in 2011 was heavily infested with mites just prior to harvest from the neighboring screen plots. Volunteer plants rapidly showed severe virus symptoms and soon died. As a result, simulated volunteer was planted again in late July. By late August few mites were present so mite populations were bolstered with mites collected from volunteer wheat collected in western Nebraska (Cheyenne County) and spread over the plots.

WCM movement was monitored in the fall of 2007, 2008, and 2010. Mite movement was evaluated around the plot area by using trap pots. Four trap pots were placed around the plots to monitor mite movement from the volunteer wheat. Each trap pot consisted of three cone-tainers (4-cm in diameter, Steuwe and Sons Inc., Tangent Oregon, USA), each cone-tainer contained three to four Millennium wheat plants. Wheat plants were grown in a greenhouse and covered with cages (5-cm in diameter and 50-cm in height) for 14 days prior to being brought to the field. Trap pots were exposed in the field for 7 days and changed weekly until late October when frost began killing the

plants. To harvest trap pots, wheat plants were cut at soil level, placed in zip-lock bags and stored at 4°C until mites were counted. Mite movement was measured by determining the percentage of trap plants with mites present.

During 2009-10 and 2010-11, mite presence in the screen was determined by randomly sampling twenty plants from the volunteer border approximately every two weeks throughout the late summer and fall to monitor mite presence and abundance. All mite counts were done under a stereo-microscope at 30X-40X to determine the number of mites on each of the plants.

Relative chlorophyll readings were taken prior to harvest summer using a SPAD-502 Chlorophyll Meter (Konica Minolta Sensing Inc.,Ramsey, NJ). An average of 10 readings were taken from each plot. A visual yellowing rating was made at the early heading stage that was based on a 0-5 scale (0 = no symptoms, 1 = some mosaic, 2 = significant mosaic, 3 = major yellowing, still green, 4 = only little green remains, 5 = yellow/brown).

At harvest the middle two rows were threshed from each plot, and the seed from each plot was then cleaned and weighed. Data were analyzed using a type 3 analysis of variance and least significant differences were used to determine significant differences between treatments (PROC MIXED; version 9.22, SAS Institute 2008).

Results

Temperature

Temperature data (Figure 4.1) were obtained for all four years from the High Plains Regional Climate Center (hprcc.unl.edu; University of Nebraska – Lincoln). Average fall temperatures in Scottsbluff, NE were higher during the 2008-09 growing season compared with the 2007-08 season. Similar differences occurred for Mead, NE with higher fall temperatures in 2010-11 compared with the 2009-10 season. A comparison of temperature between Scottsbluff (2007, 2008), and Mead (2009, 2010) indicated that spring temperatures were higher for Mead, NE compared to Scottsbluff, NE. Also, fall temperatures in 2008-09 and 2009-10 were comparable and warmer than the other years.

Mite Movement

Trap cone mite counts for October of each year indicated differences in mite activity. The accumulated percentage of plants infested with mites in October 2007 (Scottsbluff) was 16.6%; whereas, in 2008, 66.7% of plants were infested with mites. Trap cones in October 2010 (Mead) were 56% infested. No trap cones were used in 2009, but mite counts collected from sampled volunteer trap plants indicated higher mite activity than was seen in 2010.

2007/2008 Scottsbluff, NE

Data analysis between years indicated that there was a significant year by planting date and year by varieties interaction for SPAD, yellowing and yield. Therefore, each

year was analyzed separately. Relative chlorophyll (SPAD) readings (Figure 4.2) were significantly different between planting dates (F=7.17; df=2,6; P=0.0257) due to reduced average chlorophyll readings for the late planting date. Varieties were also significantly different (F=502.81; df=2,18; P=<.0001) indicating a significant virus presence. Mace (49.8) had significantly higher chlorophyll reading than Millennium (33.0), and Millennium had a significantly higher chlorophyll reading than Tomahawk (18.7). The interaction between planting date and variety was not significant (F=2.63; df=4,18 P=0.0684).

Yellowing ratings (Figure 4.3) reflected SPAD readings with similar significant differences between planting dates (F=10.33; df=2,6; P=0.0114) caused by reduced yellowing in the late planting (3.6). There were no significant differences between early (4.1) and recommended (4.0) planting dates. Significant variety differences were observed (F=54.50; df=2,18; P<.0001) with Mace (2.8) having the least yellowing, followed by Millennium (4.0) and Tomahawk (4.8). No significant interaction occurred between planting date and variety for yellowing (F=1.25; df=4,18; P=0.3256).

A significant difference in yield occurred between planting dates (F=32.51; df=2,6; P=0.0006). The recommended planting date yielded significantly more (184 kg/hectare) than early (161 kg/hectare) and late (111 kg/hectare) planting dates. Varieties were also significantly different for yield (F=19.81 df=2,18; P=<.0001). Mace (217.2 kg/hectare) yielded significantly more than Millennium (114.7 kg/hectare) or Tomahawk (114.7 kg/hectare). The interaction between planting date and variety was approaching

significance (F=2.74; df= 4,18; P=0.0818) due to increased yields in Millennium and Tomahawk between early and recommended planting dates. But in comparison, early and recommended planting had no significant impact on Mace (T=0.75; df=18; P=0.4649), while yields for the late planting dropped significantly.

2008/2009 Scottsbluff, NE

Relative chlorophyll readings increased significantly across the three planting dates (F=36.95; df=2,6; P=0.0004) (Figure 4.5). Significant variety differences (F=33.52; df=2,18; P<.0001) were seen with Mace having the greatest SPAD readings followed by Millennium and then Tomahawk. The interaction between planting date and variety approached significance (F=2.87; df=2,18; P=0.0532). This interaction resulted because Millennium and Tomahawk had similar (T=0.40 df=18; P=0.6970) and much lower SPAD readings than Mace for the first two planting dates. However, on the final planting date Millennium readings increased to close to those for Mace (T=0.44; df=18; P=0.6670) and higher than Tomahawk.

Yellowing ratings (Figure 4.6) were significant for planting dates (F=43.43; df=2,6; P=0.0003) with yellowing decreasing from early to late planting. Varieties were significantly different (F=62.00; df=2,18; P<.0001) with Mace (2.6) having significantly less yellowing than Millennium (3.8) and Tomahawk (4.4). The interaction between planting dates and varieties was significant (F=3.50; df=4,18; P=0.0278). This interaction was primarily due to Millennium having increasingly significant differences compared to Tomahawk, due to less yellowing in recommended and late planting dates.

Yields (Figure 2.7) were significantly different for planting dates (F=103.96; df=2,6; P<.0001) with late planting (237.9 kg/hectare) yielding significantly more than early (32 kg/hectare) and recommended (111 kg/hectare) planting dates. Similar differences occurred for varieties (F=34.53; df=2,18; P<.0001) with Mace (195.8 kg/hectare) yielding significantly more than Millennium (107.9 kg/hectare) and Tomahawk (78.1 kg/hectare) . There was no significant interaction between planting date and variety (F=1.33; df=4,18; P=0.2986).

2009/2010 Mead, NE

Relative chlorophyll readings were significantly different between planting dates (F=11.68; df=2,6; P=0.0085) (Figure 4.8) with a significant increase between early and the subsequent planting dates. Greater differences in SPAD readings were observed between varieties (F=356.18; df=2,18; P<.0001) with Mace (29.9) having significantly higher readings than Millennium (19.5), which was significantly higher than Tomahawk (12.3). The planting date by variety interaction was significant (F=7.13; df=4,18; P=0.0013) due to consistently greater increase in chlorophyll for Mace as the compared with decease in Millennium and minimal increase in Tomahawk over the planting dates.

Yellowing ratings were significantly different between all varieties (F=71.36; df=2,18; P=<.0001) (Figure 4.9). Mace (2.0) had significantly less yellowing than Millennium (3.0), followed by Tomahawk (3.8). There were no significant differences between planting dates (F=2.45; df=2,6; P=0.1664) and no significant interaction occurred between planting dates and varieties (F=0.96; df=4,18; P=0.4509).

Yields (Figure 4.10) resembled the differences observed in yellowing ratings. Significant differences between varieties occurred (F=75.62; df=2,18; P=<.0001), but no significant difference occurred between planting dates (F=2.05; df=2,6; P=0.2009). In the case of varieties, Mace (147.9 kg/hectare) yielded significantly more than Millennium (58.7 kg/hectare) and Tomahawk (39.0 kg/hectare). There was no planting date by variety interaction (F=1.15; df=4,18; P=0.3671).

2010/2011 Mead, NE

Relative chlorophyll readings (Figure 4.11) were significantly different for planting date (F=6.29; df=2,6; P=0.0337) with highest readings occurring in the recommended (19.3) planting date. Readings from the recommended planting date were significantly greater than the readings from the late planting (16.4) but not different than the early (17.6) planting date. Significant variety differences occurred with Mace (33.7) having significantly greater readings than Millennium (10.4) and Tomahawk (9.27) (t=14.82; df=18; P<.0001). No significant differences occurred between Millennium and Tomahawk (t=1.42; df=18; P=0.1724). A significant planting date by variety interaction (F=6.23; df=4,18; P=0.0025) occurred as a result of Mace being the only variety to have an increase in SPAD readings from the early (30.6) to the recommended (37.35) planting date. Millennium and Tomahawk had consistently low readings throughout all of the planting dates.

Significant differences in yellowing ratings (Figure 4.12) occurred for planting dates (F=5.40; df=2,6; P=0.0456) due to a decrease in yellowing in the recommended

planting date (2.3). Early (2.8) and late (2.8) had the same average yellowing rating. Varieties were all significantly different (F=41.82, df=2,18; P<.0001) with Mace (1.8) having the least yellowing, followed by Millennium (3.0) and Tomahawk (3.75). No interaction occurred between planting date and variety (F=1.06; df=4,18, P=0.4052).

Yield (Figure 4.13) was significantly different between varieties (F=83.75; df=2,18; P<.0001) due to resistant Mace (6.7 kg/hectare) being the only variety that yielded harvestable grain. Significant differences occurred between planting dates (F=8.31; df=2,6; P=0.0186) with the highest yield occurring in the recommended (3.59 kg/hectare). A significant interaction between planting date and variety (F=9.15; df=4,18; P=0.0003) occurred as result of Mace being the only variety having its highest yield in recommended (10.78 kg/hectare) compared to early (3.18 kg/hectare) and late (6.13 kg/hectare) planting dates.

Discussion

Yield impacts from the wheat-mite-virus complex varied considerably between years. Regardless of the level of impact, relative chlorophyll readings and yellowing ratings were good indicators of virus impact on yield.

Lower spring temperatures occurred during the growing seasons at Scottsbluff compared with the two at Mead. In 2007-08, a significant increase in relative chlorophyll and yield occurred between early and recommended planting dates. This was primarily due to increased yields in Millennium and Tomahawk indicating that greater virus pressure occurred on tolerant and susceptible varieties in the early planting. However, a significant yield loss in the late planting date occurred during 2007-08, but this was likely due to planting too late in the fall (10 Oct., 2007), which was followed by cooler temperatures. Late planted wheat remained green as implied by relative chlorophyll readings and yellowing rating, indicating that virus was not the primary cause of loss. These yield losses are likely due to reduced yield potential from agronomic concerns. Late planted wheat didn't have adequate time for significant tiller development prior to the onset of winter. The reduced tillering in the late planting were likely also exacerbated by lower fall temperatures that occurred during that season (Figure 4.1).

Higher fall temperatures occurred during 2008-09 compared to the 2007-08 season (Figure 4.1) and results indicated a significant increase in planting date effect for relative chlorophyll, yellowing, and yield. The differences between these two seasons indicate that fall temperature may play a critical role in the effectiveness of later planting

dates. For the resistant variety Mace there was a greater than three-fold increase in yield between early (85.35 kg/hectare) and late (302.78 kg/hectare) planting dates (Figure 2.7). Mildly tolerant Millennium (107.9 kg/hectare) and susceptible Tomahawk (78.1 kg/hectare) had similar positive responses but had significantly less yield than Mace (195.8 kg/hectare). The 2008-09 season indicates that resistant varieties in combination with delayed planting can dramatically reduce yield impact from high disease pressure situations when spring temperatures are cool.

The greatest yield loss occurred during the 2010-11 growing season at Mead, NE. Fall and spring temperatures during this season were among the highest during the four seasons of the study. During this season, susceptible varieties didn't produce any grain and resistant Mace yielded only 6.7 kg/hectare. Early (3.2 kg/hectare) and late (6.1 kg/hectare) planting dates yielded significantly less than the recommended (10.8 kg/hectare) planting date. The loss in yield in the early planting date may be a result of becoming infested with mites earlier and allowing for greater virus replication due to warmer fall temperatures. Late planting losses may be associated with reduced development of wheat during infestation and infection by virus due to high fall temperatures. Higher spring temperatures would have a significant impact on late planting due to reduced tillering and increased virus replication.

The 2009-10 season had significantly higher yields than 2010-11. Temperature comparisons between the two seasons indicate the 2009-10 had lower fall temperatures than 2010-11, but similar spring temperatures (Figure 2.1). Both studies at Mead, NE had

reduced overall yield compared with Scottsbluff, NE. The effect of planting date relative to yield gain was also greatly diminished for late planting dates at Mead, NE. The results at Mead, NE indicate that warm spring temperatures may negate the ability of late planted winter wheat to avoid significant infection and replication of viruses within the wheat-mite-virus complex. However, planting winter wheat at the recommended planting date yielded significantly more than early planting for both 2009-10 and 2010-11 growing seasons.

The severity of yield impact across all varieties and planting dates indicates that these management tactics utilized alone or in combination do not provide adequate protection under high virus potential situations. This underscores the importance of integrating alternative management tactics, such as controlling the 'green bridge', to minimize yield loss.

Mite movement data were compared with temperature, indicating greater mite movement occurring during warmer fall conditions. Increased mite movement can result in a greater number of plants becoming infested with WCM. Although trap cones were not present in the spring, significant mite movement could occur and warm temperature may allow adequate time for virus impact to occur from spring infections.

Every year of this study indicated that planting before the recommended seeding date resulted in a significant yield loss when winter wheat was under high disease pressures. Hunger *et al.* (1992) found similar results with mechanical inoculation of early and late-planted winter wheat during the fall resulting in significant yield losses due to

plants being less developed. Spring inoculation of the early planting date resulted in a minimal yield loss. Sill (1953) had concluded that wheat had to be infested in the fall when plants were young to cause significant yield impacts.

However, when considering that WCM are necessary for widespread field infections under natural conditions, planting later may avoid significant mite movement. This reduces the frequency of infested plants in late planting dates. In addition, inoculation by mites must be followed with virus replication from the point of infection for significant yield impacts to occur. In this study, maximum yield impact occurred in late planting only when high spring temperatures occurred, due to adequate temperature for virus replication. When spring temperatures were mild, later planting of winter wheat reduced the potential time for mite infestation and virus replication. Overall, this study and previous studies suggest that the stage of the wheat at the time of infestation and inoculation is critical in determining the potential for yield impact.

Of the varieties involved in the study, resistant variety Mace yielded significantly more than mildly tolerant Millennium and susceptible Tomahawk. Although Mace was the highest yielding variety, it also had a positive response in yield between early and recommended planting dates for every season of the study, demonstrating the need for integrated management options, such as planting date, even for virus-resistant varieties.

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Tables and Charts

Figure 4.1: Monthly average temperature during the winter wheat growing seasons for Scottsbluff, NE (2007/08, 2008/09) and Mead, NE (2009/10, 2010/11) (data provided by the High Plains Regional Climate Center, University of Nebraska-Lincoln).
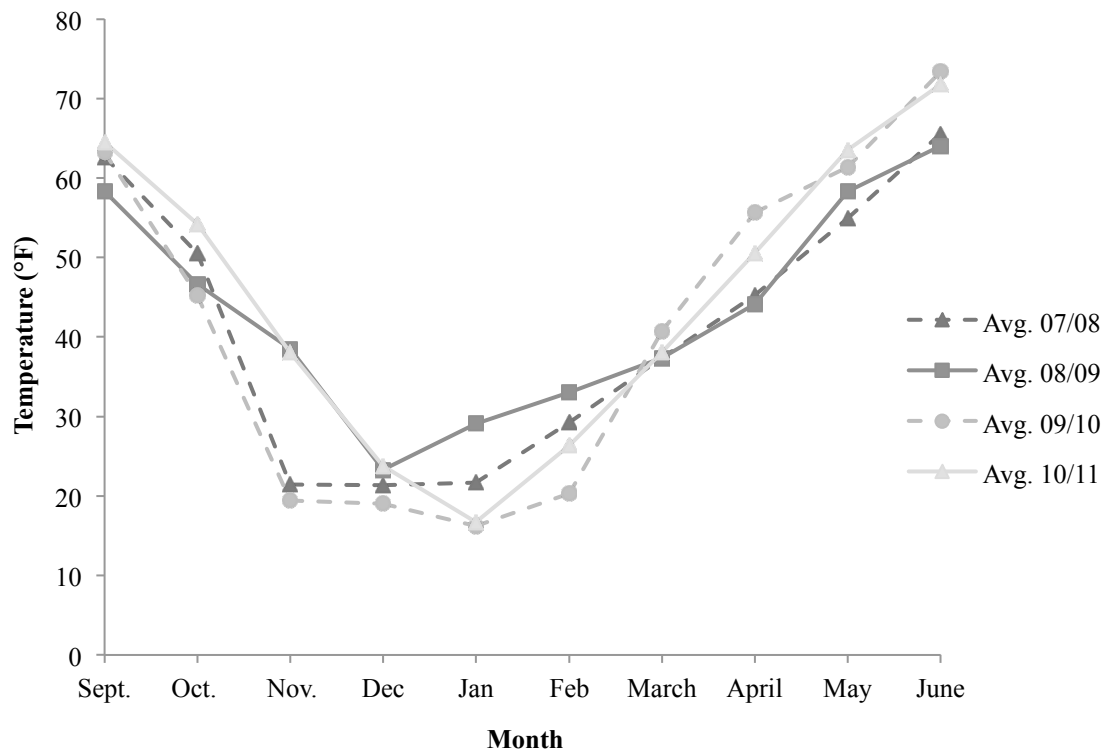
Figure 4.2: Relative chlorophyll (SPAD) readings for three winter wheat varieties across three planting dates; Scottsbluff, NE, 2007-08 (Planting date p=0.0257; Variety p<.0001; Planting date by variety p=0.0684).
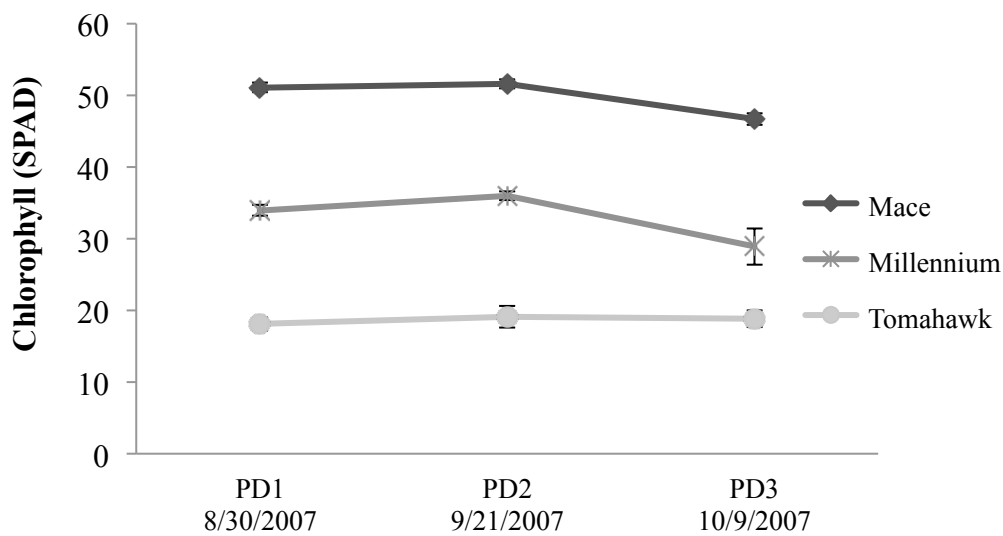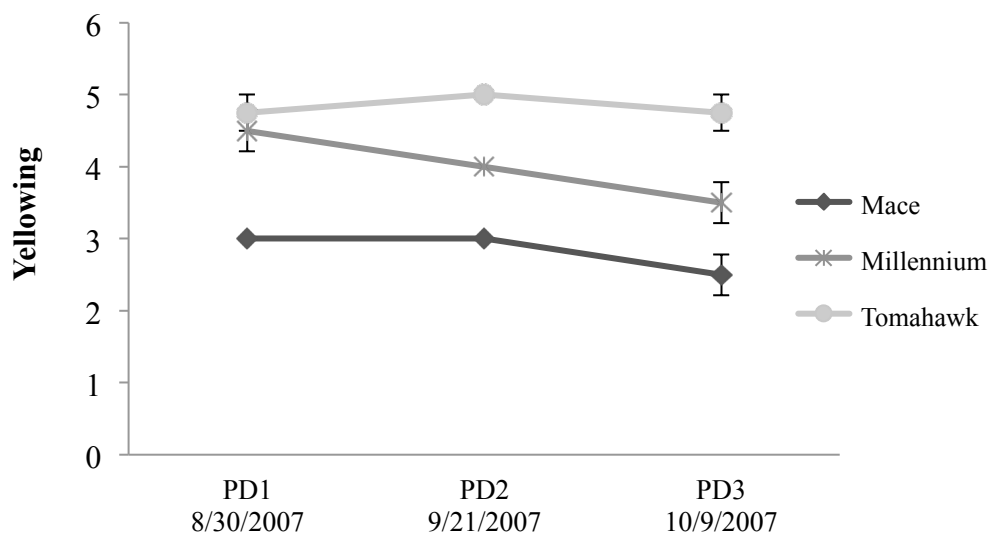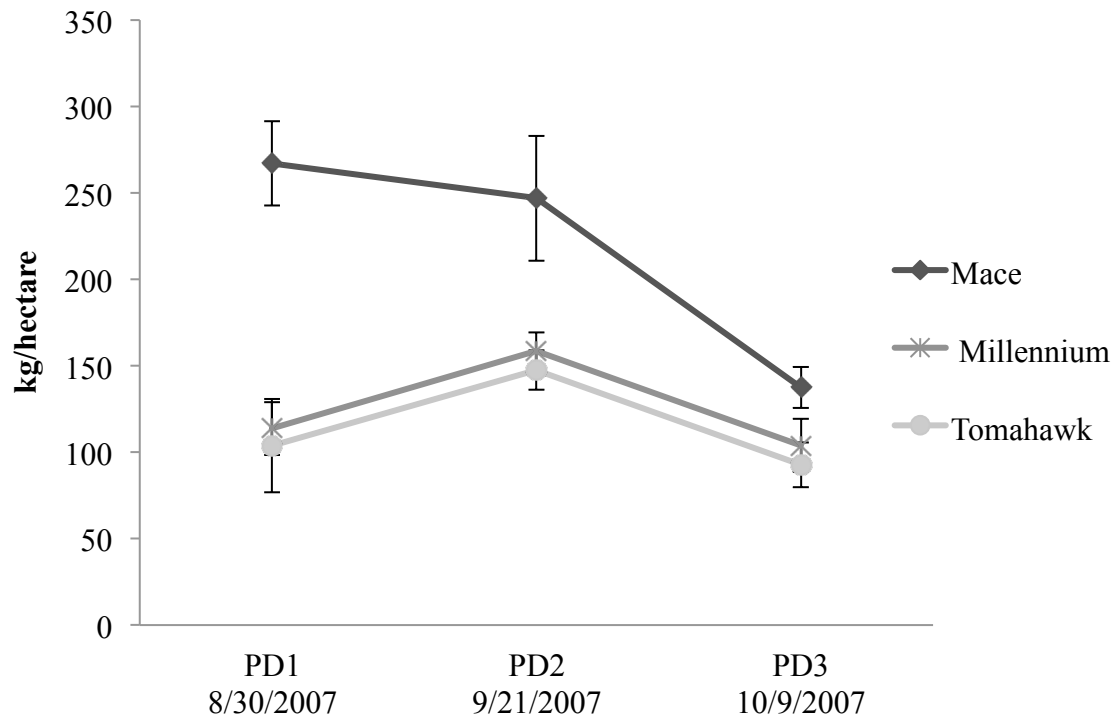

Figure 4.3: Leaf yellowing ratings (1 = healthy plant, 5 = yellow plants) for three winter wheat varieties across three planting dates; Scottsbluff, NE, 2007-08 (Planting date p=0.0114; Variety p<.0001; Planting date by variety p=0.3256).

Figure 4.4: Average yield for three winter wheat varieties across planting dates; Scottsbluff, NE, 2007-08 (Planting date p=0.0006; Variety p<.0001; Planting date by variety p=0.0818).
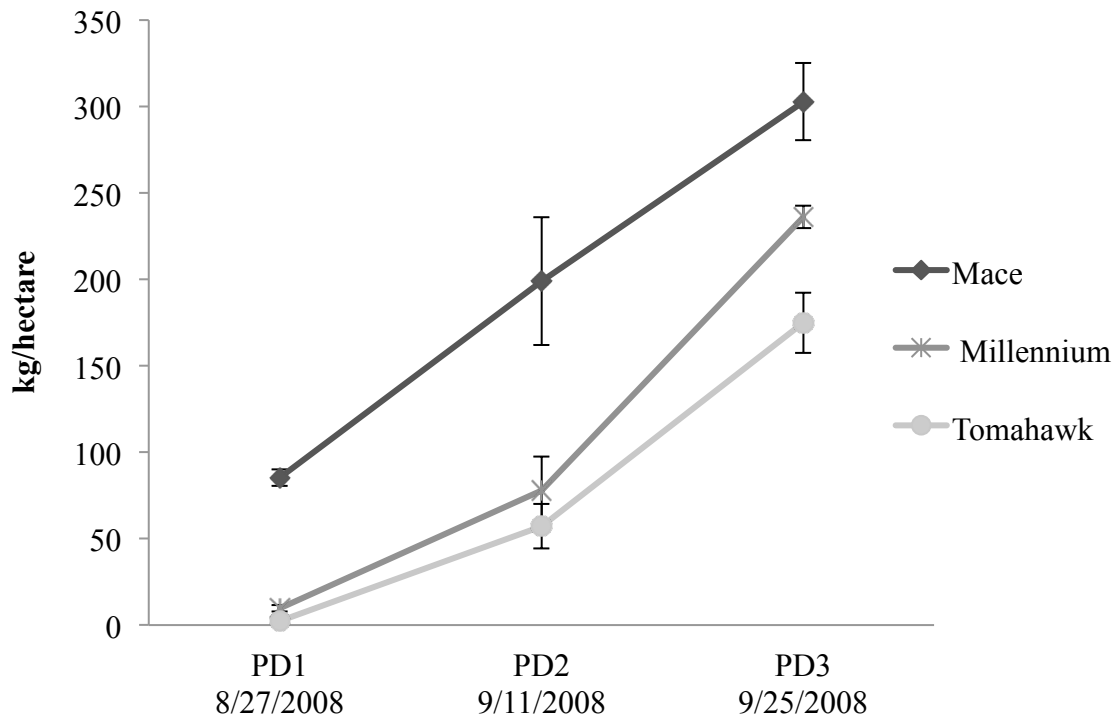
Figure 4.5: Relative chlorophyll (SPAD) readings of varieties across planting dates; Scottsbluff, NE, 2008-09 (Planting date p=0.0004; Variety p<.0001; Planting date by variety p=0.0532).
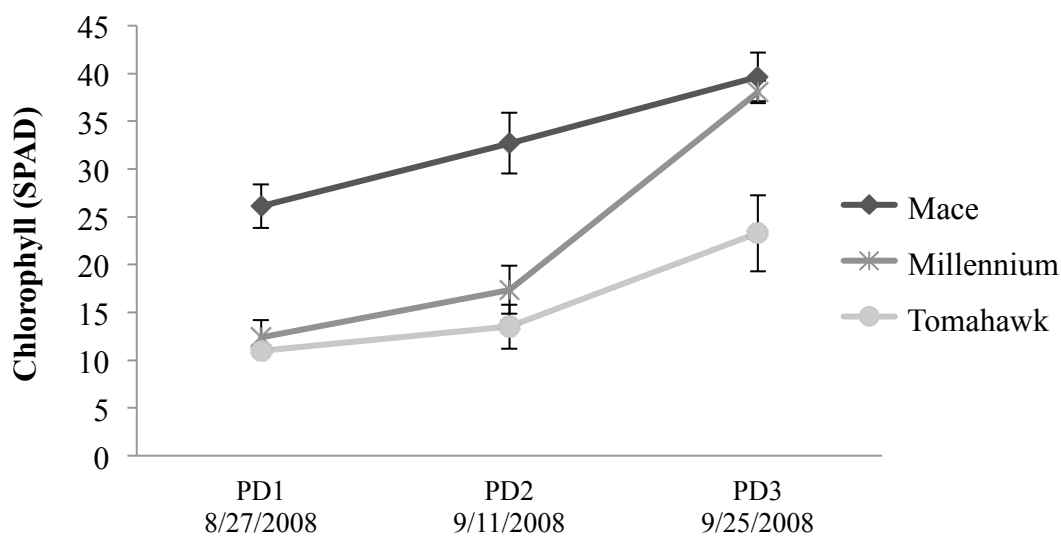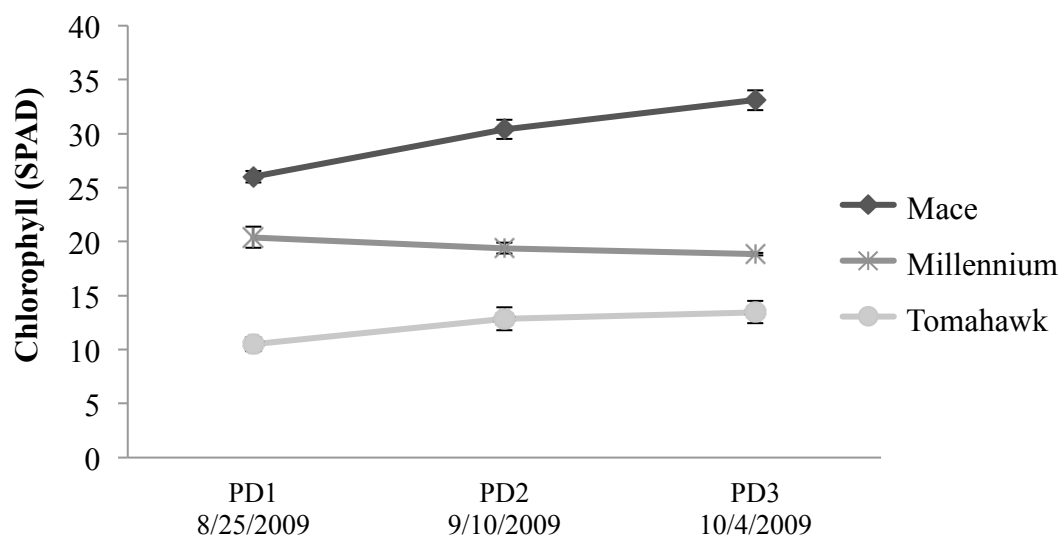

Figure 4.6: Leaf yellowing ratings of varieties across planting dates; Scottsbluff, NE, 2008-09 (1 = healthy plant, 5 = yellow plants) (Planting date p=0.0114; Variety p<.0001; Planting date by variety p=0.3256).
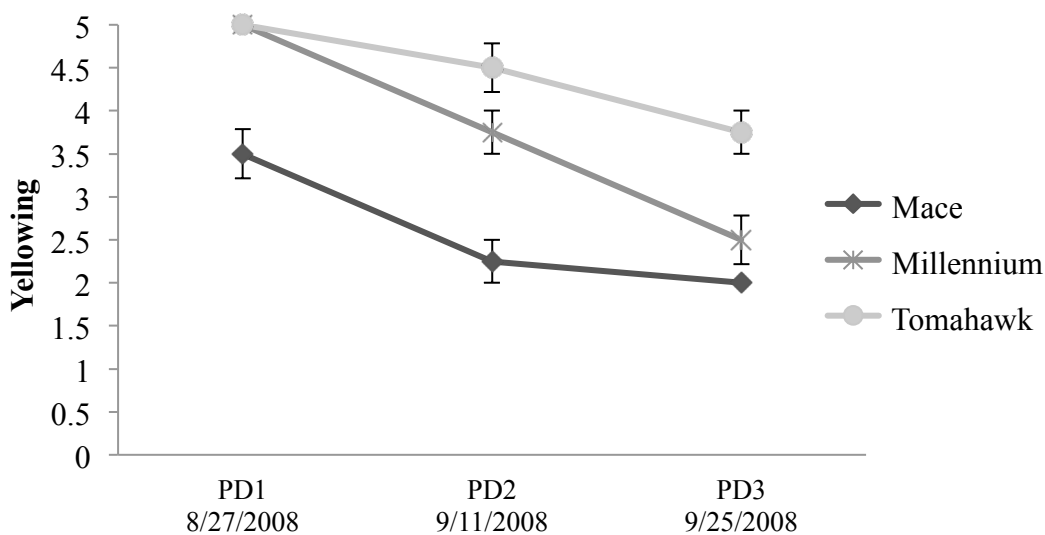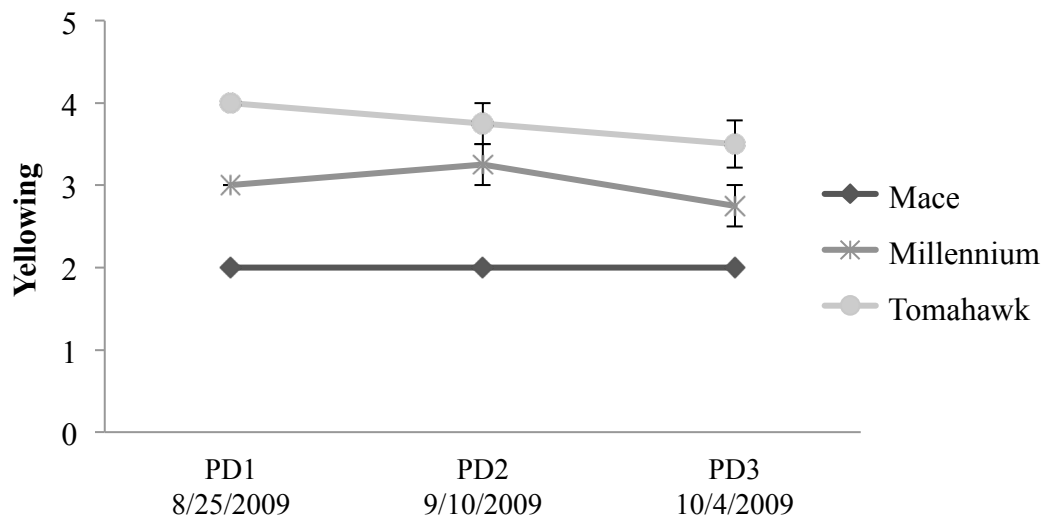
Figure 4.7: Average yield of varieties across planting dates; Scottsbluff, NE, 2008-09. (Planting date p=0.0006; Variety p<.0001; Planting date by variety p=0.0818).

Figure 4.8: Relative chlorophyll (SPAD) readings of varieties across planting dates; Mead, NE, 2009-10 (Planting date p=0.0085; Variety p<.0001; Planting date by variety p=0.0013).

Figure 4.9: Leaf yellowing ratings for varieties across planting dates; 2009-10 at Mead, NE, 2009-10 (1 = healthy plant, 5 = yellow plants) (Planting date p=0.1664; Variety p<.0001; Planting date by variety p=0.4509).

Figure 4.10: Average yield of varieties across planting dates; Mead, NE, 2009-10 (Planting date p=0.0006; Variety p<.0001; Planting date by variety p=0.0818).
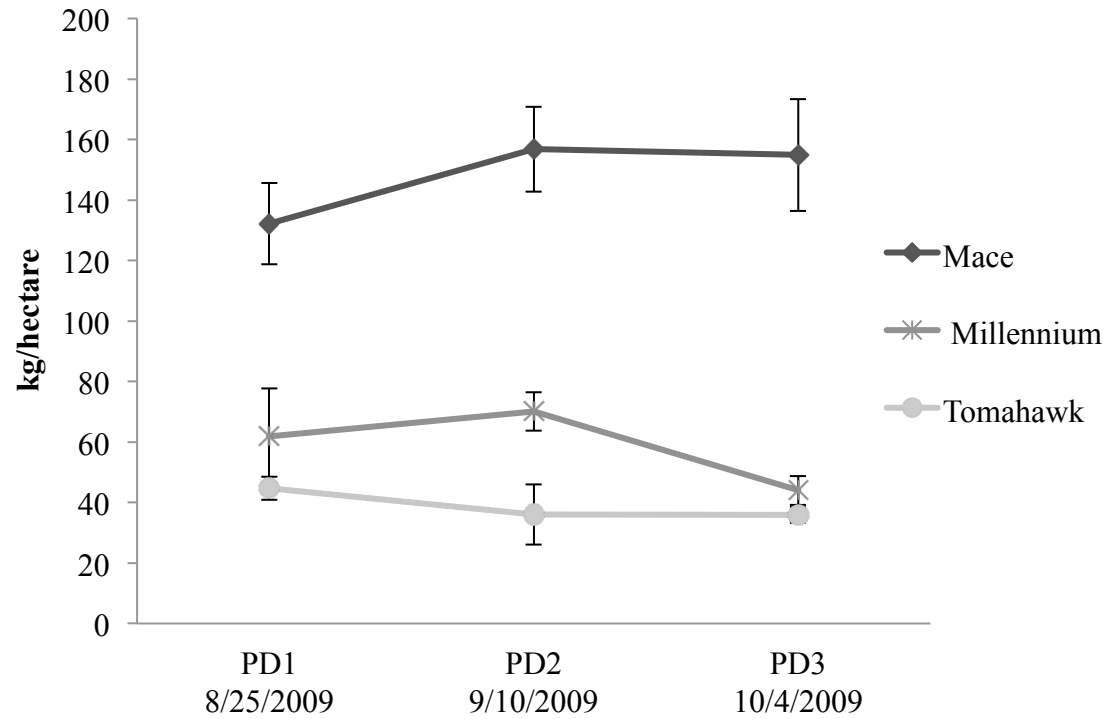
Figure 4.11: Relative chlorophyll (SPAD) readings of varieties across planting dates; Mead, NE, 2010-11. (Planting date p=0.0337; Variety p<.0001; Planting date by variety p=0.0025).

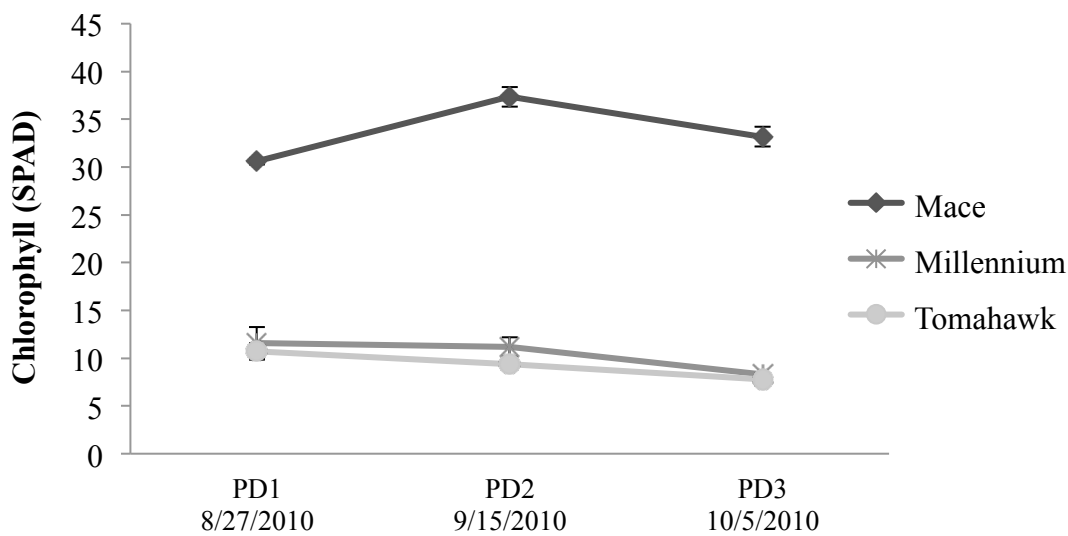**Figure 4.12: Leaf yellowing of varieties across planting dates; Mead, NE, 2010-11 (1 = healthy plant, 5 = yellow plants) (Planting date p=0.0456; Variety p<.0001; Planting date by variety p=0.4052).**
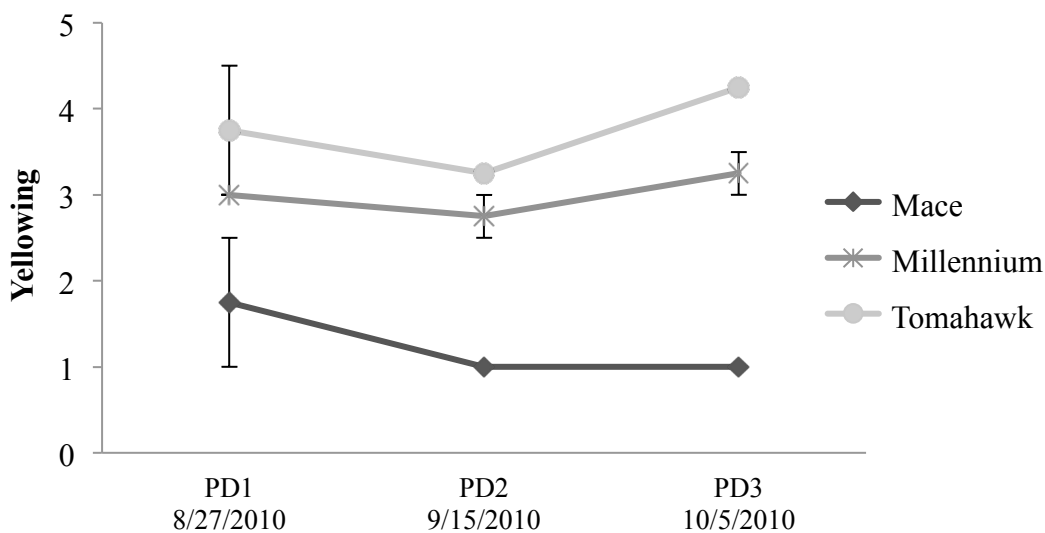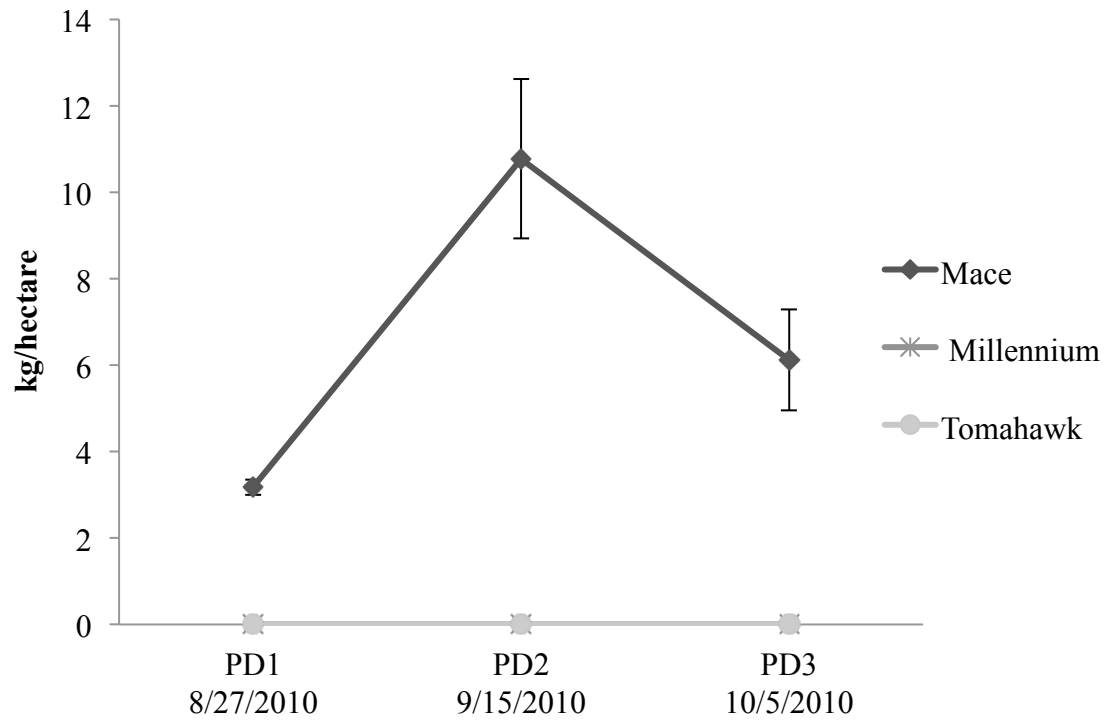
Figure 4.13: Average yield of varieties across planting dates; Mead, NE, 2010-11. Planting date p=0.0186; Variety p<.0001; Planting date by variety p=0.0003.

APPENDIX A: 10-MITE TRANSFER SAS CODE

```
data reproductive;
input code $ colony $ trt $ run rep day mite egg elisa;
datalines;

proc print;
title '10-Mite Transfer Study';
run;

ods graphics on;

/*ANOVA for comparison of main effects and interactions*/
title '10-Mite Transfer Study - Adults';

proc glimmix data=reproductive;
class colony trt rep;
model mite=colony|trt|day|day/ solution dist=negbin
htype=1;
random rep*run*colony*trt;
run;

/*ANOVA modified to contain only significant effects*/
/*Obtain solution for fixed effects to build equations*/
title '10-Mite Transfer Study - Adults';

proc glimmix data=reproductive;
class colony trt rep;
model mite=trt day(trt*colony) day*day/ noint solution
dist=negbin htype=1;
random rep*run*colony*trt;
output out=yhats pred(ilink)=p ;
run;

/*Predicted values from model*/
proc print data=yhats;
run;

/*Correlation between observed and predicted values*/
proc corr data=yhats;
var mite p;
run;
```

```
title '10-Mite Transfer Study - Eggs';

/*ANOVA for comparison of main effects and interactions*/
proc glimmix data=reproductive;
class colony trt rep;
model egg=colony|trt|day|day/ solution dist=negbin htype=1;
random rep*run*colony*trt;
nloptions maxiter=1000;
run;

title '10-Mite Transfer Study - Eggs';

/*ANOVA modified to contain only significant effects*/
/*Obtain solution for fixed effects to build equations*/
proc glimmix data=reproductive;
class colony trt rep;
model egg=trt day(trt*colony) day*day/ noint solution
dist=negbin htype=1;
random rep*run*colony*trt;
output out=yhats1 pred(ilink)=p ;
nloptions maxiter=1000;
run;

/*Predicted values from model*/
proc print data=yhats1;
run;

/*Correlation between observed and predicted values*/
proc corr data=yhats1;
var egg p;
run;
```