

Biotype composition and virulence distribution of wheat curl mite
in the North Central United States

by

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AN ABSTRACT OF A DISSERTATION

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Abstract

The wheat curl mite, *Aceria tosichella* (Keifer), is an important global pest of bread wheat, *Triticum aestivum* L. Chronic and often severe reductions of winter wheat yield due to *A. tosichella* infestations have occurred in North America and all other wheat-production areas for over five decades. Moreover, *A. tosichella* is the only vector which transmits the three most important wheat viruses in the Great Plains, which are Wheat Streak Mosaic Virus (WSMV), the most economically important wheat virus in North America; Triticum Mosaic Virus (TriMV) and High Plains Wheat Mosaic Virus (HPWMoV). Mite infestation alone causes stunted, chlorotic plants in susceptible wheat varieties. To date, mite resistant wheat cultivars have been the only sufficient method to control *A. tosichella*. The discovery of new genes for *A. tosichella* resistance and their introgression into wheat cultivars are essential steps to combat the development of new and/or different *A. tosichella* biotypes which can develop to overcome resistance genes. Both *A. tosichella* biotype 1 and 2 exist in U. S. Great Plains wheat producing areas. Elucidating and predicting *A. tosichella* population composition changes based on climatic and geographic variables is a key to continued effective mite management. Experiments were conducted to: 1) assess *A. tosichella* virulence in mites collected from 25 sample sites in six states to wheat plants harboring the *Cmc2*, *Cmc3* and *Cmc4* mite resistance genes and the *Wsm2* WSMV resistance gene in 2014 and 2015, and determine the distribution of WSMV, TriMV and HPWMoV present in mites collected; 2) assess *A. tosichella* biotype composition using internal transcribed spacer 1 (ITS1) and cytochrome oxidase I (COI) polymorphisms; 3) use generalized additive modeling to capture the spatio-temporal factors contributing to the prevalence of *A. tosichella* biotypes 1 and 2; and 4) screen Kansas advanced breeding lines for resistance to *A. tosichella* biotypes 1 and 2.

Results indicated that *A. tosichella* collected from 92% of the sample area were virulent to susceptible Jagger wheat plants with no *Cmc* resistance genes; that mites from 36% of the sample area were virulent to the *Cmc2* gene, and that mites collected from 24% of sample area were virulent to *Cmc3*. Mite populations from only 8% of the sample sites exhibited virulence to plants containing *Cmc4* + *Wsm2* or *Cmc4*. The WSMV virus was predominant and present in 76% of all mites sampled. HPWMoV and TriMV were less apparent and present in 16% and 8% of all mites sampled, respectively. These results will enable breeders to increase the efficiency of wheat production by releasing wheat varieties containing *A. tosichella* resistance genes that contribute to reducing virus transmission. Results of spatio-temporal factor modeling provide new, more accurate information about the use of ground-cover and precipitation as key predictors of biotype prevalence and ratio.

Experiments to determine if Kansas State University advanced breeding lines contain *A. tosichella* resistance found no resistance to biotype 1, resistance to biotype 2 in breeding lines AYN3-37 and AYN3-34; and moderate resistance to biotype 2 in breeding lines AYN2-28 and AYN2-36.

The demonstrated correlation between reduced *A. tosichella* population size and avirulence; characterization and prediction of the *A. tosichella* biotype composition; and the identification of new sources of *A. tosichella* resistance in wheat can help entomologists and wheat breeders increase wheat production efficiency by releasing additional wheat cultivars containing *A. tosichella* resistance genes.

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Experiments to determine if Kansas State University advanced breeding lines contain *A. tosichella* resistance found no resistance to biotype 1, resistance to biotype 2 in breeding lines AYN3-37 and AYN3-34; and moderate resistance to biotype 2 in breeding lines AYN2-28 and AYN2-36.

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Dedication

This dissertation is dedicated to my father, Kahtan Khalaf Al-Ani, a vow from me to march on the right pathway.

Chapter 1 - Introduction

Wheat, *Triticum aestivum* L., is the world's most widely grown crop, providing 20% of the daily protein and calories for more than 50% of the world population (FAOSTAT 2015).

Wheat is the second most important food crop in the developing world after rice. The demand for more wheat is expected to increase by 60% by 2050 (FAOSTAT 2015).

The wheat curl mite, *Aceria tosichella* Keifer (Order: Acari; Family: Eriophyidae), is a microscopic yellow-white arthropod with a cigar-shaped body about 200 microns in length and 75 microns wide (Del Rosario; Sill 1965). *A. tosichella* developmental stages are: egg, two nymphal instars and adult. *A. tosichella* has two quiescent periods before each molt (Staple and Allington 1956; Manson and Oldfield 1996). Nault and Styer (1969) reported that the leaf of the wheat plant protects all mite life stages, allowing them to survive through the overwinter. Townsend et al. (1996) highlighted that all life stages can survive 0°C for ~ 3 months. However, Staples and Allington (1956) reported that the development of the mite is very slow at 9°C and stops at 0°C.

Aceria tulipae, (Keifer) found on tulip bulbs, was originally described by Keifer (1938) to infest both tulip bulbs and winter wheat. However, Keifer later identified the mite on winter wheat as *A. tosichella* (Keifer 1969). Soon after, differences revealed between *A. tulipae* on Liliaceae hosts and *Aceria tritici* (Shevtchenko et al. 1970) on winter wheat.

A. tosichella is now a global eriophyid pest of wheat on all continents (Navia et al. 2010). This mite reduces wheat yields by feeding on leaf epidermal tissue (Orlob 1966; Murugan et al. 2011) causing direct damage from leaf rolling around the leaf midvein and occasional trapping of the flag leaves (Orlob 1966), and by transmission of three wheat viruses (Slykhuis 1955; Atkinson and Grant 1967; Velandia et al. 2010). *A. tosichella*-related yield losses range from 9-

30% (Harvey et al. 2000, 2002), while losses due to infection by WCM transmission of the Wheat Streak Mosaic Virus range from 2.5 to 7%, depending on weather conditions and wheat cultivar (Tosic 1971; Martin et al. 1984; Shahwan et al. 1984; Christian and Willis 1993; Mahmood et al. 1998; Seifers et al. 1996, 2011; Hunger et al. 2004; Velandia et al. 2010; Appel et al. 2015; Rotenberg et al. 2016). Additional yield loss may occur due to WCM transmission of the *High Plains Wheat Mosaic Virus* (HPWMoV, genus *Emaravirus*, formerly *High Plains virus*; www.ictvonline.org/proposals-15/2015.018aP.A.v3. Emaravirus _sp.pdf), and *Triticum Mosaic Virus* (TriMV, family Potyviridae, genus Poacevirus). Aggregate yield losses from single, double, or triple viral coinfections are prevalent in the central United States and have been shown to range from 37% (single) to 5% (triple) (Burrows et al. 2009, Byamukama et al. 2012, 2013, 2014; Mahmood et al. 1998; Seifers et al. 2011). The barley yellow dwarf virus (BYDV) is second important damaging wheat viruses, Appel et al. (2015) reported annual wheat yield loss due to BYD can be 1%.

To date, there are no effective acaricides available to manage the *A. tosichella* because the cryptic behavior the mite exhibits when hiding inside rolled leaves protects them. Although systemic acaricides might control the mite, the viruses transmitted by WCM will remain in the plant causing yield loss. In addition, the mite is very light in weight, and disperses easily from plant to plant by wind (Sabelis and Bruin 1996) and further complicating long-term chemical control (Thomas and Hein 2003). Harvey et al. (1979) reported that applying systemic chemicals to the soil at planting time could control mite infestations during the fall, but those effects are lost by spring.

In the U.S. Great Plains, volunteer wheat plants and more than 100 species of weedy grasses over-summer, serving as a green bridge to provide a host for *A. tosichella* to survive until

fall planting (Connin 1956; Somsen and Sill 1970; Harvey et al. 2002). In addition to removal of volunteer wheat plants, delayed planting has been recommended to break the green bridge and suppress *A. tosichella* populations. However, delayed planting is more normally determined by availability of soil moisture and is not feasible for growers using wheat as a winter forage (Staples and Allington 1956; Martin et al. 1984; Wegulo et al. 2008; Velandia et al. 2010).

Host plant resistance to arthropods is the most viable, economical, and environmentally-safe approach to reduce pest damage (Smith 1999, 2005). Mite-resistant wheat varieties have been shown to effectively manage *A. tosichella* and reduce wheat yield losses (Harvey and Livers 1975; Harvey et al. 1994). Research in *A. tosichella* resistance in wheat was first conducted by Andrews and Slykhuis (1956) who identified mite resistance from progeny of crosses between tall wheatgrass, *Agropyron elongatum* (Host.) P. Beauv., and *Agropyron intermedium* (Host.) P. Beauv., to bread wheat. Harvey and Livers (1975) determined that genes from rye, *Secale cereale* L., suppress mite populations more than genes from wheat.

Martin et al. (1976) reported that Salmon (*Cmc3*), a wheat cultivar carrying a segment of rye chromosome 1R, was highly resistant to *A. tosichella* but not to WSMV. However, a later study by Martin et al. (1984) reported resistance in Salmon reduced the occurrence of WSMV by 58%. Harvey and Martin (1980) and Harvey et al. (1990) highlighted that increased trichome density in some wheat cultivars reduced *A. tosichella* populations. Several wheat cultivars with genetic resistance to *A. tosichella* have been used effectively to reduce *A. tosichella* populations resulting in less infection by mite-vectored viruses (Harvey and Martin 1988; Harvey et al. 1994, 2005; Conner et al. 1991; Malik et al. 2003; Murugan et al. 2011; Carver et al. 2016; Chuang et al. 2017; Aguirre-Rojas et al. 2017).

Because *A. tosichella* transmits WSMV, researchers found a source of resistance to WSMV represented by the *Wsm1* gene, which was transferred from intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth and D. R. Dewey) (Wells et al. 1973; 1982; Triebe et al. 1991; Gill et al. 1995). Soon after, Seifers et al. (2007) identified *Wsm2* in the CO960293-2 wheat germplasm resulting in the variety RonL. Both genes are good sources of resistance to WSMV in areas that have temperatures less than 24°C after fall planting (Seifers et al. 2006). However, *Wsm1* is ineffective above 18°C and *Wsm2* is ineffective above 24°C (Seifers et al. 2006; Liu et al. 2011; Kumssa et al. 2017)

Harvey et al. (1997, 1999) reported that *A. tosichella* biotype 1 is avirulent to *Cmc3* and biotype 2 is virulent to the same gene. Smith (1999) indicated that the selection pressure on pest populations can limit deploying resistance genes that may result in development of virulent pest biotypes. That was extremely true in the case of releasing the first commercial *A. tosichella*-resistant wheat cultivar TAM 107 in the late 1980's, which contained *Cmc3* which originated from a rye translocation into wheat *A. tosichella* (Sebesta and Wood 1978; Thomas and Conner 1986). TAM 107 (*Cmc3*) significantly suppressed mite populations and their associated viruses until results by Harvey et al. (1997, 1999) indicated that resistance in TAM 107 had been overcome by some mite populations.

Biological strains of insects with physiological differences within a species that allow individuals to survive on plants with different resistance genes, is a term originally identified by Painter (1951). Since, such strains have come to be referred to as biotypes. Many factors are involved with biotype development, including the type of selection pressure applied to the arthropod population by the plant, the genetics of the host plant, and the genetics of the arthropod (Gallun 1972). Slykhuis (1955) was the first to demonstrate that *A. tosichella* has more than one

biotype on wheat. Harvey et al. (1995a) was the first to report *A. tosichella* biotype development in response to different wheat genes for mite resistance. Following that, Harvey et al. (1995b, 1999, 2001) and Malik et al. (2003) showed repeatedly that *A. tosichella* populations from different geographic locations in North America differ in their virulence responses to wheat resistance genes, while Seifers et al. (2002) emphasized that mite populations differ in their ability to vector WSMV and HPWMoV. Skoracka and Kuczynski (2006) elucidated morphological differences in *A. tosichella* populations in Poland.

Malik (2001) initially determined U.S. biotypes 1 and 2 using an internal transcribed spacer 1 (ITS1) region. Based on that finding, Siriwetwivat (2006) and Hein et al. (2012) in the U.S.; and Carew et al. (2009) and Schiffer et al. (2009) in Australia confirmed these results. Schiffer et al. (2009) also showed that WSMV is transmitted only by *A. tosichella* biotype 2 in Australia. Recently, Skoracka et al. (2012, 2013) and Szydło et al. (2015) distinguished at least eight genetic lineages in an *A. tosichella* complex in Poland and Turkey.

Based on that, we hypothesized that different geographical regions likely have different types of *A. tosichella*, which was very clearly shown in the responses of 25 different mite populations in the current study to different *Cmc* resistance genes in wheat plants. Therefore, we conducted additional evaluations of *A. tosichella* responses to Kansas wheat advanced breeding lines to identify potentially new resistance genes. Specimens used in this research are deposited as voucher number 249 in the KSU Museum of Entomology and Prairie Arthropod Research.

The goals of this study were to obtain new knowledge for more effective IPM programs of *A. tosicHELLa*. In order to do that four research projects were conducted. These included:

1. Assess the virulence of 25 mite populations collected from wheat in the Central U.S. to wheat genotypes containing *Cmc2*, *Cmc3*, *Cmc4*, and *Cmc4 + Wsm2*.
2. Determine the presence of WSMV, HPWMoV, and TriMV in mites from each *A. tosicHELLa* geographic population and the ability of each to transmit each virus into wheat plants.
3. Assess the current genetic variation and distribution of *A. tosicHELLa* biotypes 1 and 2 in 25 counties of 6 states based on ITS1 polymorphism and use spatio-temporal models to predict the prevalence of each biotype.
4. Assess potential resistance in a group of the Kansas advanced breeding lines to *A. tosicHELLa* biotypes 1 and 2.

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Chapter 2 - Changes in Virulence of North American *A. tosichella*

Populations to Mite Resistance Genes in Wheat

Abstract

Severe winter wheat yield losses due to infestations of wheat curl mite, *Aceria tosichella* Keifer, and mite-transmitted viruses occur in wheat production areas of the United States and Canada. Mite infestation alone causes stunted, chlorotic plants in susceptible wheat varieties, and mites transmit Wheat Streak Mosaic- (WSMV), High Plains Wheat Mosaic- (HPWMoV), and Triticum Mosaic Virus (TriMV). Wheat curl mites were collected from 25 sites in Kansas, Missouri, Nebraska, Texas, North Dakota and South Dakota in 2014 and 2015. At each site, mite virulence was determined to wheat plants harboring the *Cmc2*-, *Cmc3*-, or *Cmc4* mite resistance gene; or *Cmc4* plus the *Wsm2* WSMV-resistance gene. Mites collected from 92%, 36% and 24% of sites were virulent to susceptible Jagger wheat plants (no *Cmc*), *Cmc2*, and *Cmc3*, respectively. The mega-population consisting of all 25 mite sub-populations was avirulent to 80% of plants containing *Cmc4* + *Wsm2* and *Cmc4*. WSMV, HPWMoV, or TriMV was present in mites at 76%, 16% and 8% of the 25 sites, respectively. Our results will enable breeders to increase the efficiency of wheat production by releasing wheat varieties containing wheat curl mite resistance genes that reduce wheat yield losses.

Keywords: wheat curl mite, virulence, distribution, *Cmc* genes, winter wheat.

Introduction

The wheat curl mite, *Aceria tosichella* Keifer, is an important arthropod pest of winter wheat, *Triticum aestivum* L. in the central United States (Slykhuis 1955, Nault and Styer 1970). Wheat curl mite feeding reduces wheat yields by 9 to 30% (Harvey et al. 2000, 2002). However, mites are critical for infecting and moving viruses between wheat plants (Slykhuis 1955, Atkinson and Grant 1967, Velandia et al. 2010). Wheat curl mite nymphs acquire *Wheat streak mosaic virus* (WSMV, family *Potyviridae*, genus *Tritimovirus*), after feeding on infected plants for as little as 30 min and can disseminate the virus for at least 7 d post-acquisition (Slykhuis 1955, Orlob 1966, Seifers et al. 1997, 2008, 2009b, Appel et al. 2015). Yield losses from WSMV infection range from 2.5 to 7%, depending on weather conditions and wheat cultivar (Tosic 1971, Martin et al. 1984, Shahwan et al. 1984, Christian and Willis 1993, Mahmood et al. 1998, Seifers et al. 1996, 2011, Hunger et al. 2004, Velandia et al. 2010, Appel et al. 2015, Rotenberg et al. 2016).

Mites also transmit *High Plains wheat mosaic virus* (HPWMoV, genus *Emaravirus*, formerly *High Plains virus*; www.ictvonline.org/proposals-15/2015.018aP.A.v3. Emaravirus _sp.pdf), and *Triticum Mosaic Virus* (TriMV, family *Potyviridae*, genus *Poacevirus*). Aggregate yield losses from single, double, or triple viral coinfections are prevalent in the central United States (Mahmood et al. 1998, Burrows et al. 2009, Seifers et al. 2011, Byamukama et al. 2012, 2013, 2014).

Although predicting wheat curl mite infestations is possible if pre-harvest volunteer wheat or green corn are near winter wheat emerging in the fall, controlling infestations difficult and complicated because many species of range grasses also serve as mite hosts (Slykhuis 1955, Connin 1956, Gibson 1957, Somsen and Sill 1970, Skoracka et al. 2012, Velandia et al. 2010). In addition, delaying wheat planting until volunteer wheat is problematic, because the uniformity of

volunteer wheat destruction by producers varies widely. Furthermore, planting dates are normally determined by availability of soil moisture and delayed planting is not feasible for growers using wheat for winter forage (Martin et al. 1984; Velandia et al. 2010). Finally, acaricides are ineffective for *A. tosichella* management (Townsend and Johnson 1996; Morgan et al. 2005; McMechan and Hein 2016).

Thus, wheat plant resistance is likely to be the most viable, economical, and environmentally-safe and eco-friendly approach to reduce wheat curl mite occurrence (Smith 1999). Andrews and Slykhuis (1956) were the first to identify mite resistance in progeny from crosses involving tall wheatgrass, *Agropyron elongatum* (Host.) P. Beauv., and *Agropyron intermedium* (Host.) P. Beauv., to bread wheat. Harvey and Livers (1975) determined that genes from rye, *Secale cereale* L., suppress mite populations more than genes from wheat, and Martin et al. (1976) showed that Salmon, a wheat cultivar carrying a segment of rye chromosome 1R, was highly resistant to wheat curl mite but susceptible to WSMV. Soon after, Martin et al. (1984) highlighted that resistance to wheat curl mite in Salmon reduced the occurrence of WSMV by 58%. Harvey and Martin (1980) and Harvey et al. (1990) found that cultivars with increased trichome density had reduced wheat curl mite populations. Numerous wheat cultivars with genetic resistance to mites have been used successfully to suppress mite populations and lower the level of infection of mite-vectored viruses by inhibiting mite reproductive capacity (Harvey and Martin 1988, Harvey et al. 1994, 2005, Conner et al. 1991).

Four cereal genes expressing resistance to wheat curl mite have been deployed in commercial varieties in North America. *Cmcl* from Tausch's goatgrass, *Aegilops tauschii* (Coss.) Schmal. (syn. *Ae. squarrosa* L.; *Triticum tauschii*) was introgressed to wheat chromosome 6D (Thomas and Conner 1986, Whelan and Thomas 1989); in wheat cultivar

Radiant (Thomas et al. 2012). *Cmc4* in wheat cultivars MT06X424 (Hofer et al. 2011) and OK05312 (Carver et al. 2016), also from *Ae. tauschii*, was shown to segregate independently of *Cmc1* on wheat chromosome 6D by Malik et al. (2003). *Cmc2* (PI52452) originated from *A. elongatum* and was identified by Martin et al. (1976) and Whelan and Hart (1988); and *Cmc3*, the rye gene in wheat cultivars Salmon and TAM107 (Martin et al. 1983, Schlegel and Kynast 1987), was mapped and named by Malik et al. (2003). The *Wsm1*, *Wsm2*, and *Wsm3* WSMV resistance genes have been identified (Triebe et al. 1991, Liu et al. 2011, Lu et al. 2011), but no HPWMoV resistance genes exist. Currently, no known commercial wheat varieties combine resistance to wheat curl mite and WSMV (Graybosch et al. 2009, DeWolf et al. 2014).

The term virulence in studies of plant resistance to arthropods is defined as the ability of an arthropod to overcome a plant resistance gene (Smith 2005, Kobayashi 2016, O'Neal et al. 2018) or conversely, the loss of a resistant plant's capability to distinguish the presence of the arthropod, due to mutation in an arthropod avirulence gene(s) (Smith and Clement 2012). The limitation of using any *Cmc* gene is that virulent wheat curl mite populations may preexist or develop to overcome resistance (Harvey et al. 1995, 1997, 1999). Thus, new sources of mite resistance must be available to wheat breeding programs to manage wheat curl mite virulence effectively. Mite virulence patterns in North America have not been examined since 1998. The goals of this study were to assess the virulence of mite populations collected from wheat in the Central U.S. to wheat genotypes containing *Cmc2*, *Cmc3*, *Cmc4*, and *Cmc4 + Wsm2*; and to determine the distribution of WSMV, HPWMoV, and TriMV harbored by mites collected from different geographic populations. We hypothesized that all Central U.S. mite populations have remained avirulent to *Cmc4* (that *Cmc4* has remained resistant), that virulence to *Cmc2* and *Cmc3* has developed (genotypes with *Cmc2* and *Cmc3* have become susceptible), and that

distribution of wheat curl mite-transmitted viruses has changed since 1998. The focus of the experiments conducted was to compare the reaction of genotypes containing the *Cmc2*, *Cmc3*, *Cmc4*, and *Wsm2* genes to each of 25 different wheat curl mite populations in the Central United States.

Materials and Methods

Wheat curl mites were collected from grain heads of wheat, *Triticum aestivum* L., at 25 locations in the U.S. Great Plains from May 21 to July 10, 2014, and June 25 to July 12, 2015 (Supplemental Table 1). The selection of locations was based on results of Harvey et al. (1995, 1997, 1999) and known high wheat production areas (USDA NASS 2013). Thirty wheat heads were sampled from each of three fields at each location (the distance between fields was within several hundred meters), resulting in a total of 90 heads per location to have a good representation of each wheat curl mite population. Ten wheat heads (~1000 mites/head) from each location were arbitrarily selected for establishing a mite colony for each population. Mites from each location were placed separately on susceptible Jagger plants in 45 × 45 × 75 cm mite-proof cages (BioQuip, Rancho Dominguez, CA, USA) covered with 36 µm mesh. In all experiments, Sungrow METRO-MIX 360 soil was used (Hummert International, Topeka, KS, USA) to grow wheat varieties and planted one seed per pot. After three weeks, each colony was checked under the microscope to make sure the transfer was successful. An 8 h recess was observed between transfers between each population to prevent cross-contamination between mite populations (Orlob 1966). After each colony was established, a nuclear ribosomal internal transcribed spacer 1 (ITS1) marker (Malik 2001) was used to verify that each colony contained only *A. tosichella* and was free of contamination of other *Aceria* spp.

Experiments were conducted to assess differences in mite populations on plants containing different wheat curl mite resistance genes, differences in mite-related leaf rolling in the same plants, and to determine the degree of infection of each mite population by Wheat Streak Mosaic Virus (WSMV), High Plains Wheat Mosaic Virus (HPWMoV), and Triticum Mosaic Virus (TriMV) (Seifers et al. 2009a). All known commercial wheat varieties lack combined resistance to WSMV and wheat curl mite (DeWolf et al. 2014; Graybosch et al. 2009). Three wheat genotypes with different *Cmc* genes were assessed for wheat curl mite virulence: PI52452, which contains *Cmc2*; TAM 107, which contains *Cmc3*; and advanced Kansas breeding line KSU2R-2, containing *Cmc4* and *Wsm2*. The pedigree of KSU2R-2 is RonL/U5287//KS06O3A ~58. The cultivars Jagger and OK05312, containing *Cmc4* (Carver et al. 2016) were included as susceptible and resistant controls, respectively. Plants of all these wheat genotypes tested in all experiments were maintained in the greenhouse at 24:20°C day/night and a 14:10 [L:D] h photoperiod.

Separate experiments were conducted with each of the 25 mite populations described in Supplemental Table 1. Each experiment contained a total of 25 plants, consisting of five two-leaf-stage seedlings (replications) of each of the three test genotypes, the OK05312 resistant cultivar and the Jagger susceptible control. Each seedling was infested in the second leaf stage with a piece of wheat leaf containing 10 wheat curl mite female adults and all 25 plants were caged for 14 d in 45 × 45 × 75 cm mite-proof cages (BioQuip, Rancho Dominguez, CA, USA) covered with 36 µm mesh. Plants were arranged in a completely randomized design. Each cage was rotated 90° southward daily to make sure each plant inside each cage had the same opportunity to receive sunlight and airflow.

After assessment for leaf folding (binary measure) (Chuang et al. 2017), all plants were cut just above the soil level and their leaves were spread on adhesive 5 × 9 cm gridded blue paper sheets. Each sheet was then stored in a 50 mL Falcon tube (Fisher Scientific, Waltham, MA, USA) for 4-5 d or until the leaves dried. All tubes were kept at room temperature (Murugan et al. 2011) and placed in a tube holder at a 45° angle to prevent mites from falling into the bottom of the tube. Mites migrated from leaves to adhesive and were counted using a Nikon SMZ-645 stereo zoom microscope at 50X magnification.

Fifteen two-leaf-stage seedlings of the mite-susceptible cultivar Jagger were each infested in the second leaf stage with a piece of wheat leaf containing 10 female adults from mite populations collected from each of the 25 locations. Plants were caged for 21 d for potential virus infection (Chuang et al. 2017). Each cage represented a separate experiment with each mite population. Five plants from each group of 15 infested seedlings and two negative control plants were subjected to ELISA (Seifers 1992, 2008, 2009b; Louie et al. 2006) for WSMV, HPWMoV or TriMV, respectively. Absorbance values at 405 nm were measured after 60 min, using a Vmax kinetic microplate reader (Molecular Devices, San Francisco, CA, USA) and used to calculate virus infection rates in plants fed on by mites from each location. Samples were determined to be positive for a virus if they contained three times the average absorbance values of un-infected Jagger leaves (Fahim et al. 2012; Rotenberg et al. 2016). Six un-infested seedlings of the susceptible cultivar Jagger (two plants for each of the three viruses) were caged as negative controls for calculating an ELISA ratio, which were defined as: $OD_{405\text{ nm}}$ value of infected leaf/ $OD_{405\text{ nm}}$ value of uninfected control leaf, where a high ratio indicates disease susceptibility and a low ratio indicates resistance (Fahim et al. 2012, Seifers et al. 2009a).

Mite populations infesting plants that exhibited an ELISA ratio significantly higher than plants infested by other mite populations were considered to have a significantly greater infection capacity (the percentage of infected plants) for each virus. The virus threshold value was the highest ELISA value calculated from 150 from virus-free non-infected control plants. This group of controls consisted of two control plants used to test for mite-vectored infection by mites from each of 25 locations for the three viruses.

Independent statistical comparisons were made with mites from the population collected at each of the 25 locations (in Supplemental Table 1) for differences in leaf folding, mite population counts and virus infection in plants containing the *Cmc2*, *Cmc3*, or *Cmc4* plus *Wsm2* resistance genes and resistant and susceptible controls. Data were independently analyzed for each of these three variables. Leaf folding data were analyzed by comparing each of the wheat curl mite-resistant genotypes *Cmc2*, *Cmc3*, or *Cmc4* + *Wsm2* to resistant- and susceptible control cultivars using the χ^2 Fisher's Exact Test (Fisher 1954). If the χ^2 test was significant at $p < 0.05$ for the complete experiment, individual statistical treatment differences were displayed as significant (0.05), highly significant (0.01) or non-significant at 0.05. Mite population and virus infection (ELISA ratio) data were subjected to one-way ANOVA using PROC GLIMMIX (SAS 2008). These data did not follow assumptions of normality and homogeneity of variances as indicated by the Shapiro-Wilk test of normality, and the Brown-Forsythe and Levene tests of homogeneity of variances (Shapiro and Francia 1972). Thus, both mite population count- and virus ELISA ratio data were fit to gamma distributions after transformed with log function for analysis. Mean \pm 95% CI numbers of mites and mean \pm 95% CI ELISA ratios were separated by Tukey's HSD (honestly significant difference) test or least significant difference (LSD) test if the type III test for fixed effect was significant at $P < 0.05$. The LSD test, recommended for

exploratory studies by Milliken and Johnson (2004) such as these, was conducted when the Tukey's HSD test was too conservative.

Results

Plant leaf folding responses

Mites within each of the 25 populations caused variable responses in phenotypic leaf folding to mite resistant- and susceptible control plants. Feeding by mites from 15 of the 17 locations resulted in 0-20% leaf folding on OK05312 resistant control plants and 80-100% leaf folding on susceptible Jagger control plants (Tables 2-4). However, there were exceptions to this pattern in mites from Dickinson, Finney and Geary counties, Kansas, which caused only 40-60% leaf folding on the susceptible control; and in mites from Pettis and Pike county Missouri, which caused 0-40% leaf folding on the resistant control and 60-80% folding on the susceptible control.

In Kansas plants containing *Cmc4* and *Wsm2* sustained significantly less leaf folding than susceptible Jagger control plants when infested with mites from Barton, Ellis, Ellsworth, Greeley, and Saline counties (Table 1). However, there were no statistical differences between resistant and susceptible controls and all three genotypes tested when fed on by mites from Dickinson, Finney and Geary counties, Kansas. Plants containing *Cmc2* demonstrated significantly less leaf folding than susceptible Jagger control plants when infested with mites from Greeley County; and plants containing *Cmc3* demonstrated a similar response when infested with mites from Barton and Greeley counties. There were no significant differences in leaf folding between resistant- and susceptible control plants or plants containing any of the resistance genes infested by any other Kansas mite population with the exception of five populations on plants containing *Cmc4* + *Wsmv2* (Table 1).

In Missouri, the Cooper County population caused significantly less folding to plants containing *Cmc2*, *Cmc3*, or *Cmc4 + Wsmv2* than susceptible control plants (Table 2). Feeding by populations in Cape Girardeau and Stoddard counties also caused significantly less folding in *Cmc4 + Wsmv2* plants than in susceptible control plants (Table 2). The Barton County population caused significantly less folding in *Cmc3* plants, but not to plants containing *Cmc2* or *Cmc4 + Wsmv2*. There were no significant differences in leaf folding between resistant- and susceptible control plants or plants containing any of the resistance genes infested with mite populations from Pettis and Pike counties, Missouri.

Leaf folding by all Nebraska and South Dakota mite populations was significantly less on plants containing *Cmc4* and *Wsm2* than on susceptible plants and folding was no different than that on resistant plants (Table 3). Leaf folding caused by mites in Hughes County, South Dakota, was significantly less on plants containing *Cmc2* and *Cmc3* than the susceptible plants, and folding caused by mites in Tripp County, South Dakota, was significantly less on plants containing *Cmc3* than on susceptible plants (Table 3). Mite populations in North Dakota and Texas caused significantly less folding to plants containing *Cmc4 + Wsmv2* than the susceptible (Table 4), and both North Dakota populations caused significantly less folding to plants containing *Cmc3*.

Mite Population Counts

The mean mite populations at 14 dpi differed significantly between *Cmc4* resistant- and susceptible control plants when infested with 20 of the 25 mite populations (Table 5). However, there were a range of responses to resistance genes in populations from Ward County, North Dakota, Dickinson and Greeley counties, Kansas, and Cape Girardeau and Cooper counties, Missouri. The Ward County population was avirulent to the susceptible, and populations from

Cape Girardeau, Cooper, Dickinson and Greeley counties, were avirulent to the *Cmc4* resistant control (Table 5). Populations of mites from Dickinson County, Kansas, did not differ on any plant genotype and were relatively reduced (16.6 - 68.6 mites/plant) (Table 5). The numbers of mites from Cape Girardeau and Cooper counties, Missouri, were significantly less on plants containing *Cmc3* than susceptible controls, and populations of mites from Cape Girardeau were significantly lower on plants containing *Cmc3* or *Cmc4 + Wsm2* than susceptible controls (Table 5). Mites from Ward County, North Dakota, exhibited a unique response (no differed significantly between *Cmc4* resistant- and susceptible control). Finally, mite populations on plants containing *Cmc4*, *Cmc4 + Wsm2* and the susceptible control did not differ, and all were significantly less than those on plants containing *Cmc2* or *Cmc3* (Table 5).

Cmc2 was the least resistant of all genes assessed, with mites from only 6 of 25 locations exhibiting populations on *Cmc2* plants that were significantly lower than those on susceptible control plants. The breadth of resistance exhibited by *Cmc3* plants was somewhat better, where mites from 13 of 25 locations in Kansas, Missouri, Nebraska, North Dakota, South Dakota, and Texas exhibiting populations on *Cmc3* plants that were significantly lower than those on susceptible control plants (Table 5). Plants containing the *Cmc4 + Wsm2* combination displayed the greatest level of resistance of all genotypes tested. Mite counts on *Cmc4 + Wsm2* plants were significantly lower than those on susceptible control plants when infested with mites from 20 of 25 locations, including all locations in Nebraska, South Dakota, Texas, five of the six Missouri locations, and five of the eight Kansas locations (Table 5).

To summarize Table 5, plants containing *Cmc4 + Wsm2* and *Cmc4* exhibited the highest resistance to the 25 wheat curl mite populations. The majority (80%) of the populations were susceptible to *Cmc4 + Wsm2* and were susceptible to plants containing *Cmc4*. Conversely, 92%

of the 25 populations were virulent to susceptible Jagger plants; while 40% and 24% of all populations were virulent to *Cmc2* plants, and *Cmc3* plants, respectively.

Mite Virulence

The responses of mites from all 25 populations to *Cmc4* showed no indication of wide-scale mite virulence to *Cmc4* (Table 6, Figure 1). However, counts of mites from Greeley and Dickinson counties Kansas, and Cape Girardeau and Cooper counties Missouri did not differ significantly on *Cmc4* resistant- and susceptible control plants, suggesting the potential existence of virulence to *Cmc4* at these locations. The overall pattern of mite virulence was very similar to the trends in mite population abundance shown in Table 5.

Virus Infection

Among all 25 mite populations, WSMV was the most prevalent virus, present in 76% of the sampled populations. HPWMoV and TriMV were much less prevalent, present in only 16% and 8% of the populations, respectively. WSMV was also present at significantly greater levels than HPWMoV or TriMV in mites collected from 19 of the 25 locations (Figures 2-5; Supplementary Tables 2 and 3). In addition, ELISA ratios of all three viruses were below control plant threshold levels in mites from Geary and Saline counties Kansas; Pettis County, Missouri; and Saunders and Furnas County Nebraska. ELISA ratios of HPWMoV were below control plant threshold levels in mites from all counties except Lake and Tripp, South Dakota; and ELISA ratios of TriMV were under control plant threshold levels in mites from all locations except Barton and Pike counties, Missouri (Figures 2-5). Mites collected from Bottineau County, North Dakota; and Lake and Tripp counties, South Dakota, exhibited WSMV and HPWMoV ELISA ratios no different in each paired combination within a population, but were significantly greater than the TriMV ELISA ratio. Mites collected from Ward County, North Dakota, contained a HPWMoV

ELISA ratio significantly greater than the WSMV or TriMV ratios (Supplementary Table 2, Figures 3 and 4). TriMV was present only in mites collected from Barton and Pike counties, Missouri (Figure 5).

Table 2-1. *A. tosichella*- induced leaf folding in plants of wheat genotypes containing the *Cmc2* or *Cmc3* *A. tosichella* resistance genes, *Cmc4* plus the *Wsm2* Wheat Streak Mosaic Virus resistance gene, the OK05312 *Cmc4* resistant control and the Jagger susceptible control at 14 d post infestation by *A. tosichella* populations from eight counties in Kansas.

	χ^2 Fisher's exact test															
	Barton		Dickinson		Ellis		Ellsworth		Finney		Geary		Greeley		Saline	
Control plant rating	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
% Leaf folding	0	100	0	40	0	100	0	100	0	60	0	40	20	100	0	80
Genotype [Resistance gene(s)]																
PI525452 / <i>Cmc2</i>	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
TAM 107 / <i>Cmc3</i>	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
KSU2R-2 / <i>Cmc4</i> + <i>Wsm2</i>	ns	**	ns	ns	ns	**	ns	**	ns	ns	ns	ns	ns	**	ns	*

R = *Cmc4* resistant control (OK05312); S = susceptible control (Jagger).

* significant at $P < 0.05$; ** significant at $P < 0.01$; ns = non-significant at $P > 0.05$.

Table 2-2. *A. tosichella*- induced leaf folding in plants of wheat genotypes containing the *Cmc2* or *Cmc3* *A. tosichella* resistance genes, *Cmc4* plus the *Wsm2* Wheat Streak Mosaic Virus resistance gene, the OK05312 *Cmc4* resistant control and the Jagger susceptible control at 14 d post infestation by *A. tosichella* populations from six counties in Missouri.

Control plant rating % Leaf folding	χ^2 Fisher's exact test											
	Barton		Cape Girardeau		Cooper		Pettis		Pike		Stoddard	
	R	S	R	S	R	S	R	S	R	S	R	S
	0	80	0	80	0	100	0	60	40	80	0	100
Genotype [Resistance gene(s)]												
PI525452 / <i>Cmc2</i>	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
TAM 107 / <i>Cmc3</i>	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
KSU2R-2 / <i>Cmc4</i> + <i>Wsm2</i>	ns	ns	ns	*	ns	**	ns	ns	ns	ns	ns	**

R = *Cmc4* resistant control (OK05312); S = susceptible control (Jagger).

* significant at $P < 0.05$; ** significant at $P < 0.01$; ns = non-significant at $P > 0.05$.

Table 2-3. *A. tosicHELLa*- induced leaf folding in plants of wheat genotypes containing the *Cmc2* or *Cmc3* *A. tosicHELLa* resistance genes, *Cmc4* plus the *Wsm2* Wheat Streak Mosaic Virus resistance gene, the OK05312 *Cmc4* resistant control and the Jagger susceptible control at 14 d post infestation by *A. tosicHELLa* populations from Nebraska and South Dakota.

	χ^2 Fisher's exact test													
	Nebraska county								South Dakota county					
	Saunders		Hayes		Furnas		Cheyenne		Hughes		Tripp		Lake	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
% Leaf folding	0	100	0	100	0	100	0	100	0	100	0	80	0	80
Genotype [Resistance gene(s)]														
PI525452 / <i>Cmc2</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
TAM 107 / <i>Cmc3</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	*	ns	ns
KSU2R-2 / <i>Cmc4</i> + <i>Wsm2</i>	ns	*	ns	**	ns	**	ns	**	ns	**	ns	*	ns	*

R = *Cmc4* resistant control (OK05312); S = susceptible control (Jagger). *significant at $P < 0.05$;

** significant at $P < 0.01$; ns = non-significant at $P > 0.05$.

Table 2-4. *A. tosichella*- induced leaf folding in plants of wheat genotypes containing the *Cmc2* or *Cmc3* *A. tosichella* resistance genes, *Cmc4* plus the *Wsm2* Wheat Streak Mosaic Virus resistance gene, the OK05312 *Cmc4* resistant control and the Jagger susceptible control at 14 d post infestation by *A. tosichella* populations from North Dakota and Texas.

	χ^2 Fisher's exact test							
	North Dakota county				Texas county			
	Bottineau		Ward		Dallam		Randall	
Control plant rating	R	S	R	S	R	S	R	S
% Leaf folding	0	100	0	100	0	80	0	80
Genotype [Resistance gene(s)]								
PI525452 / <i>Cmc2</i>	ns	ns	*	ns	*	ns	ns	ns
TAM 107 / <i>Cmc3</i>	ns	**	ns	**	ns	ns	ns	ns
KSU2R-2 / <i>Cmc4</i> + <i>Wsm2</i>	ns	**	ns	*	ns	*	ns	*

R = *Cmc4* resistant control (OK05312); S = susceptible control (Jagger).

* significant at $P < 0.05$; ** significant at $P < 0.01$; ns = non-significant at $P > 0.05$.

Table 2-5. Mean \pm CI (95%) number *A. tosichella* on plants of wheat genotypes containing *Cmc2* or *Cmc3* *A. tosichella* resistance genes, *Cmc4* plus the *Wsm2* Wheat Streak Mosaic Virus resistance gene, the OK05312 *Cmc4* resistant control and the Jagger susceptible control at 14 d post infestation by *A. tosichella* populations from 25 counties in Kansas, Missouri, Nebraska, North Dakota, South Dakota and Texas.

Sample location		Mean \pm CI (95%) number of <i>A. tosichella</i> on plants with mite resistance gene(s)				
State	County	None	<i>Cmc2</i>	<i>Cmc3</i>	<i>Cmc4+Wsm2</i>	<i>Cmc4</i>
KS	Barton	50.2 \pm (28.3,72.0) ab	33.8 \pm (11.9,55.6) abc	58.0 \pm (36.1,79.8) a	9.0 \pm (-12.8,30.8) bc	5.8 \pm (-16.0,27.6) c
	Dickinson	68.6 \pm (23.3,201.9) a	68.4 \pm (23.2,201.3) a	43.6 \pm (14.8,128.3) a	16.6 \pm (5.6,48.8) a	17.2 \pm (5.8,50.6) a
	Ellis	156.8 \pm (109.5,204.0) a	98.8 \pm (51.5,146.0) ab	30.4 \pm (-16.8,77.6) b	9.8 \pm (-37.4,57.0) b	27.6 \pm (-19.6,74.8) b
	Ellsworth	687.6 \pm (243.6,1940.4) a	46.8 \pm (16.5,132.0) b	244.6 \pm (86.6,690.2) ab	59.0 \pm (20.9,166.5) b	55.4 \pm (19.6,56.3) b
	Finney	27.6 \pm (15.8,47.9) b	60.6 \pm (34.8,105.3) ab	91.1 \pm (52.4,158.5) a	7.4 \pm (4.2,12.8) c	3.2 \pm (1.8,5.5) c
	Greeley	206.2 \pm (72.4,587.0) a	12.4 \pm (4.3,35.3) c	120.4 \pm (42.2,342.7) ab	23.6 \pm (8.2,67.1) bc	29.4 \pm (10.3,83.7) abc
	Geary	246.0 \pm (109.5,552.3) a	69.0 \pm (30.7, 154.9) ab	46.1 \pm (20.5,103.7) bc	12.4 \pm (5.5,27.8) c	10.8 \pm (4.8,24.2) c
	Saline	50.2 \pm (28.4,71.9) ab	33.8 \pm (12.0,55.5) abc	58.0 \pm (36.2,79.7) a	9.0 \pm (-12.7,30.7) bc	3.6 \pm (-18.1,25.3) c
MO	Barton	617.4 \pm (320.6, 1188.7) a	530.8 \pm (275.6, 1022.0) ab	66.7 \pm (34.6, 128.6) c	86.2 \pm (44.7, 165.9) c	153.6 \pm (79.7, 295.7) bc
	Cape Girardeau	473.6 \pm (311.6, 635.5) a	255.2 \pm (93.2, 417.1) ab	16.0 \pm (-145.9, 177.9) b	130.2 \pm (-31.7, 292.1) b	153.6 \pm (-8.3, 315.5) ab
	Cooper	404.8 \pm (143.4, 1141.9) a	87.6 \pm (31.0, 247.1) ab	25.8 \pm (9.1, 72.7) b	153.2 \pm (54.3, 432.1) ab	108.4 \pm (38.4, 305.8) ab
	Pike	1510.6 \pm (1068.1, 2136.3) a	1091.0 \pm (771.4, 1542.9) a	284.6 \pm (201.2, 402.4) b	244.6 \pm (172.9, 345.9) b	264.8 \pm (187.2, 374.4) b
	Pettis	227.4 \pm (123.3, 419.2) a	10.4 \pm (5.6, 19.1) b	4.3 \pm (2.3, 8.1) bc	2.0 \pm (1.0, 3.6) c	1.8 \pm (0.9, 3.3) c

Table 2-5. continued

Sample location		Mean \pm CI (95%) number of <i>A. tosicHELLa</i> on plant with mite resistance gene(s)				
State	County	None	<i>Cmc2</i>	<i>Cmc3</i>	<i>Cmc4+Wsm2</i>	<i>Cmc4</i>
	Stoddard	228.8 \pm (105.2, 497.4) a	168.8 \pm (77.6, 366.9) a	17.8 \pm (8.1, 38.6) b	8.6 \pm (3.9, 18.6) b	33.9 \pm (15.6, 73.9) b
NE	Cheyenne	457.2 \pm (199.4, 1048.3) a	113.6 \pm (49.5, 260.4) abc	193.6 \pm (84.4, 443.9) ab	31.2 \pm (13.6, 71.5) c	59.2 \pm (25.8, 135.7) bc
	Furnas	1616.2 \pm (665.6, 3924.0) a	653.2 \pm (269.0, 1585.9) a	338.2 \pm (139.3, 821.1) ab	98.0 \pm (40.3, 237.9) b	72.4 \pm (29.8, 175.7) b
	Hayes	1034.6 \pm (417.2, 2565.4) a	39.0 \pm (15.7, 96.7) b	497.6 \pm (200.6, 1233.8) a	6.2 \pm (2.5, 15.3) b	17.0 \pm (6.8, 42.1) b
	Saunders	2567.4 \pm (1648.6, 3998.0) a	2393.0 \pm (1536.6, 3726.5) a	559.4 \pm (359.2, 871.1) b	218.0 \pm (139.9, 339.4) c	155.0 \pm (99.5, 241.3) c
ND	Bottineau	141.6 \pm (58.6, 341.9) a	30.8 \pm (12.7, 74.3) ab	3.4 \pm (1.4, 8.2) c	8.8 \pm (3.6, 21.2) bc	7.4 \pm (3.0, 17.8) bc
	Ward *	98.6 \pm (-50.0, 247.2) c	379.2 \pm (230.5, 527.8) a	312.4 \pm (163.7, 461.0) ab	109.4 \pm (-39.2, 258.0) bc	81.6 \pm (-67.0, 230.2) c
SD	Hughes	63.8 \pm (37.0, 110.0) a	7.4 \pm (4.2, 12.7) b	8.6 \pm (4.9, 14.8) b	5.0 \pm (2.8, 8.6) b	9.0 \pm (5.2, 15.5) b
	Lake	94.2 \pm (43.0, 206.0) a	4.6 \pm (2.1, 10.0) b	13.8 \pm (6.3, 30.1) b	3.4 \pm (1.5, 7.4) b	3.2 \pm (1.4, 6.9) b
	Tripp	183.8 \pm (77.4, 436.3) a	35.0 \pm (14.7, 83.0) ab	4.0 \pm (1.6, 9.4) c	9.8 \pm (4.1, 23.2) bc	28.4 \pm (11.9, 67.4) b
TX	Dallam	1187.8 \pm (619.0, 2279.2) a	696.6 \pm (363.0, 1336.6) ab	524.6 \pm (273.9, 1006.6) abc	248.2 \pm (129.3, 476.2) bc	151.6 \pm (79.0, 290.9) c
	Randall	468.0 \pm (255.7, 856.5) a	386.2 \pm (211.0, 706.8) a	325.6 \pm (177.9, 595.9) a	86.0 \pm (46.9, 157.3) b	46.2 \pm (25.2, 84.5) b

Means within a row followed by the same letter are not significantly different ($P > 0.05$, Tukey's mean separation test).

* Means within a row followed by a different letter differed significantly based on LSD mean separation test ($\alpha = 0.05$).

Table 2-6. Virulence of *A. tosichella* in Kansas, Missouri, Nebraska, North Dakota, South Dakota and Texas to wheat genotypes containing *Cmc2*, *Cmc3*, or *Cmc4* *A. tosichella* resistance genes, the *Wsm2* Wheat Streak Mosaic Virus resistance gene plus *Cmc4*, the OK05312 *Cmc4* mite-resistant control, and the susceptible Jagger, based on numbers of *A. tosichella* per plant at 14 d post infestation.

		Genotype and resistance gene(s)				
State	County	Jagger (<i>none</i>)	PI525452 (<i>Cmc2</i>)	TAM107 (<i>Cmc3</i>)	KSU2R-2 (<i>Cmc4+Wsm2</i>)	OK05312 <i>Cmc4</i>
KS	Barton	V	I	V	I	AV
	Dickinson	I	I	I	I	I
	Ellis	V	I	AV	AV	AV
	Ellsworth	V	AV	I	AV	AV
	Finney	V	V	V	AV	AV
	Greeley	V	AV	I	AV	I
	Geary	V	V	AV	AV	AV
	Saline	V	I	V	I	AV
MO	Barton	V	I	AV	AV	AV
	Cape Girardeau	I	I	AV	AV	I
	Cooper	V	V	AV	V	V
	Pike	V	V	AV	AV	AV

Table 2-6. continued

Genotype and resistance gene(s)						
State	County	Jagger (<i>none</i>)	PI525452 (<i>Cmc2</i>)	TAM107 (<i>Cmc3</i>)	KSU2R-2 (<i>Cmc4+Wsm2</i>)	OK05312 <i>Cmc4</i>
	Pettis	V	AV	AV	AV	AV
	Stoddard	V	V	AV	AV	AV
NE	Cheyenne	V	I	I	AV	AV
	Furnas	V	V	I	AV	AV
	Hayes	V	AV	V	AV	AV
	Saunders	V	V	I	AV	AV
ND	Bottineau	V	I	AV	AV	AV
	Ward	V	V	V	V	V
SD	Hughes	V	AV	AV	AV	AV
	Lake	V	AV	AV	AV	AV
	Tripp	V	I	AV	AV	AV

Table 2-6. continued

State	Genotype and resistance gene(s)					
	County	Jagger (<i>none</i>)	PI525452 (<i>Cmc2</i>)	TAM107 (<i>Cmc3</i>)	KSU2R-2 (<i>Cmc4+Wsm2</i>)	OK05312 <i>Cmc4</i>
TX	Dallam	V	V	I	AV	AV
	Randall	V	V	V	AV	AV

V (virulence) = mean numbers of mites on treatment plants significantly greater than or not significantly different from those on susceptible Jagger control plants.

I (intermediate) = no significant differences between mean numbers of mites on treatment plants or resistant or susceptible control plants.

AV (avirulence) = mean numbers of mites on treatment plants significantly less than or not significantly different from those on resistant OK05312 control plants.

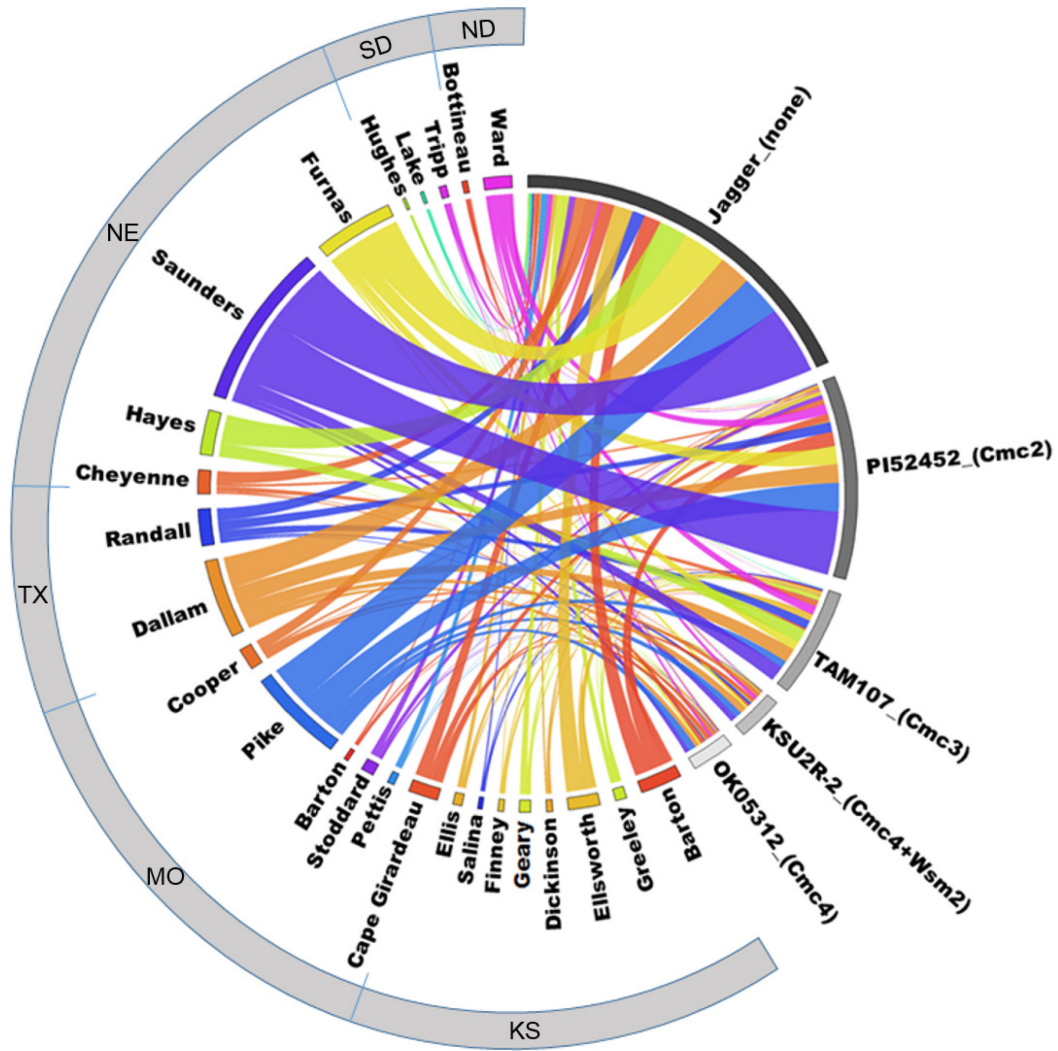


Figure 2-1. Virulence of *A. tosichella* on wheat genotypes containing *Cmc2*, *Cmc3*, or *Cmc4* *A. tosichella* resistance genes, the *Wsm2* Wheat Streak Mosaic Virus resistance gene plus *Cmc4*, the OK05312 *Cmc4* mite-resistant control and the susceptible Jagger control at 14 dpi by *A. tosichella* populations collected from 25 counties in six U. S. Great Plains wheat producing states. Color bands represent *A. tosichella* numbers from each population on plants of each genotype. Narrow bands indicate avirulence, wide bands indicate virulence.

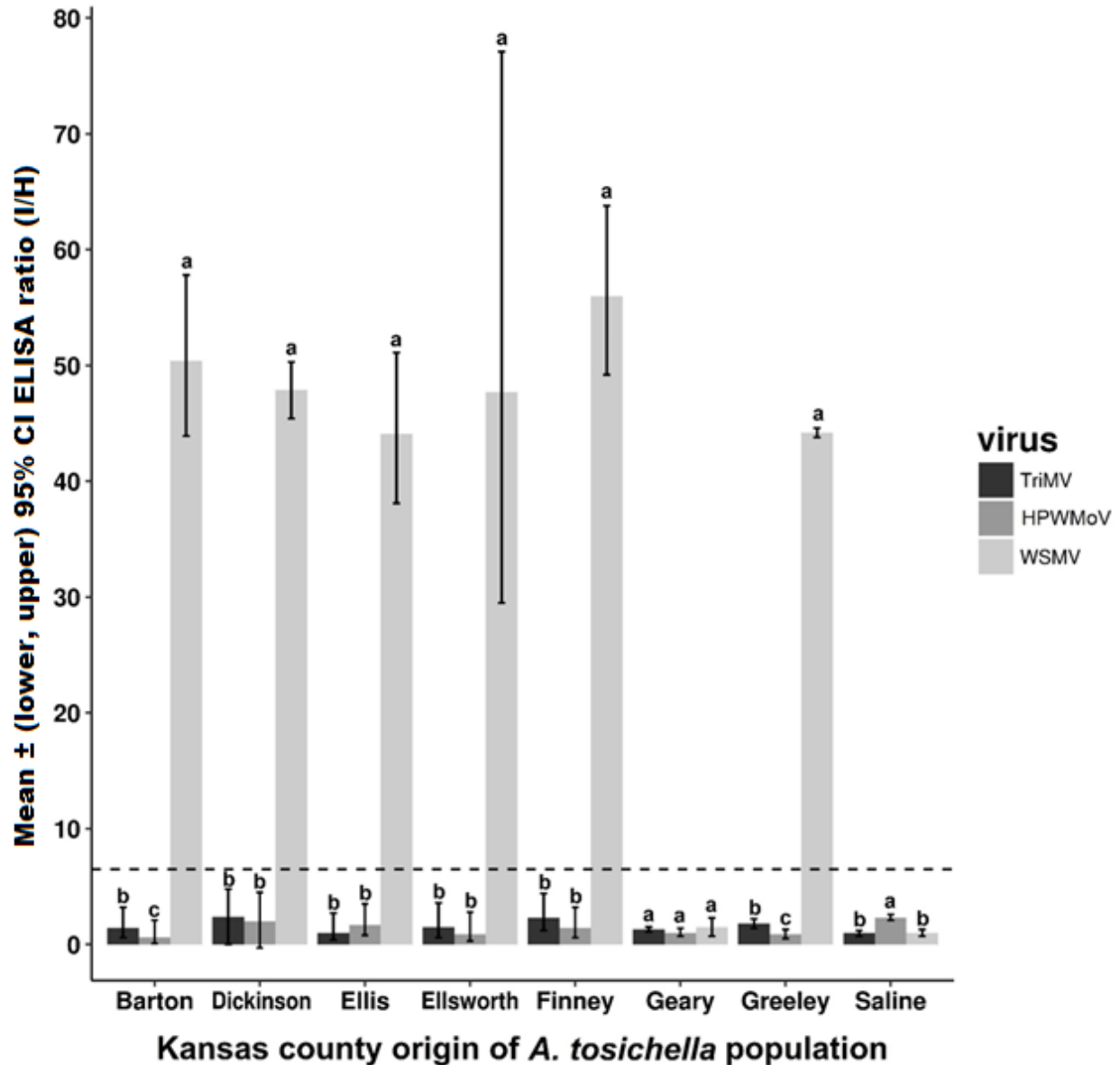


Figure 2-2. Mean \pm (lower, upper) 95% CI ELISA ratios for *Wheat Streak Mosaic Virus* (WSMV), *High Plain Wheat Mosaic Virus* (HPWMoV) and *Triticum Mosaic Virus* (TriMV) detected in *A. tosichella* collected at eight locations in Kansas after 21 d of feeding on plants of the *A. tosichella*-susceptible Jagger wheat. a, b- Means followed by a different letter at each location differ significantly at $p < 0.05$ (Tukey's HSD test). ELISA ratio = (OD₄₀₅ value of infected leaf/OD₄₀₅ value of healthy uninfected leaf). Dashed line = maximum ELISA threshold ratio for virus-free control plants.

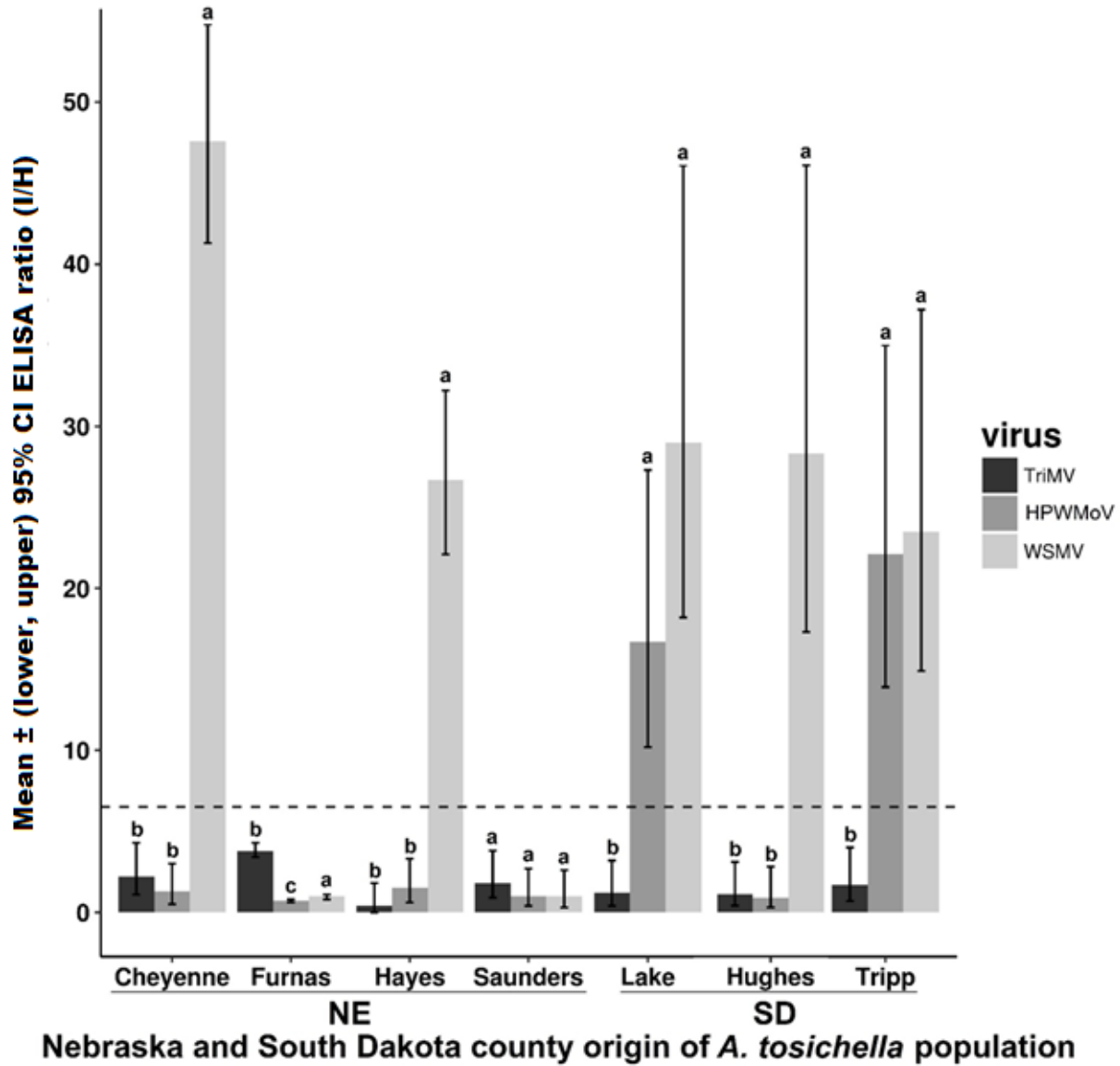


Figure 2-3. Mean \pm (lower, upper) 95% CI ELISA ratios for *Wheat Streak Mosaic Virus* (WSMV), *High Plain Wheat Mosaic Virus* (HPWMoV) and *Triticum Mosaic Virus* (TriMV) detected in *A. tosicHELLa* at four locations in Nebraska and three locations in South Dakota after 21 d of feeding on plants of the *A. tosicHELLa*-susceptible Jagger wheat. a, b- Means followed by a different letter at each location differ significantly at $p < 0.05$ (Tukey's HSD test). ELISA ratio = (OD₄₀₅ value of infected leaf/OD₄₀₅ value of healthy uninfected leaf). Dashed line = maximum ELISA threshold ratio for virus-free control plants.

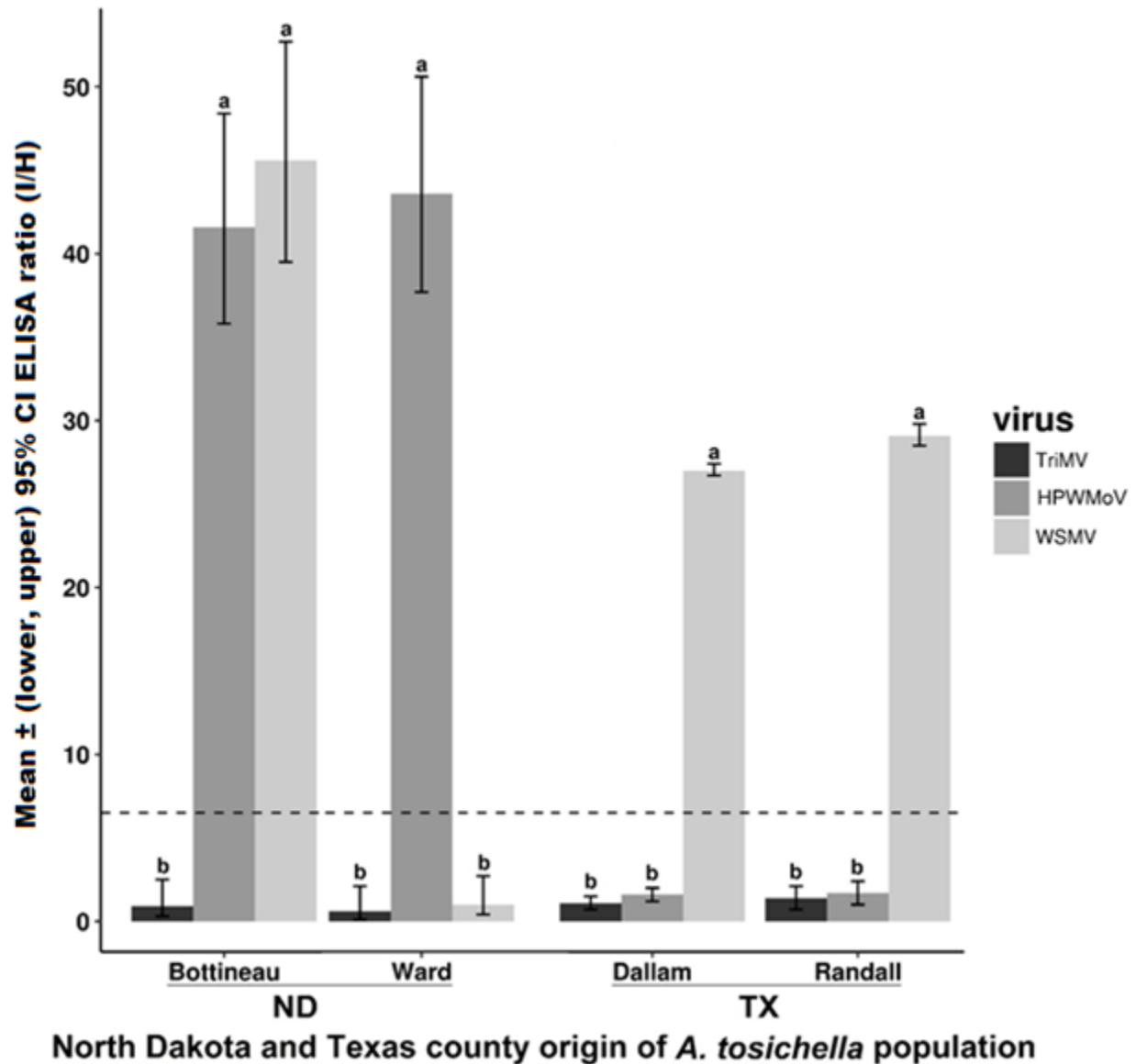


Figure 2-4. Mean \pm (lower, upper) 95% CI ELISA ratios of *Wheat Streak Mosaic Virus* (WSMV), *High Plain Wheat Mosaic Virus* (HPWMoV) and *Triticum Mosaic Virus* (TriMV) detected in *A. tosichella* collected at two locations in North Dakota and two locations in Texas after 21 d of feeding on plants of the *A. tosichella*-susceptible Jagger wheat. a, b- Means followed by a different letter at each location differ significantly at $p < 0.05$ (Tukey's HSD test). ELISA ratio = (OD₄₀₅ value of infected leaf/OD₄₀₅ value of healthy uninfected leaf). Dashed line = maximum ELISA threshold ratio for virus-free control plants.

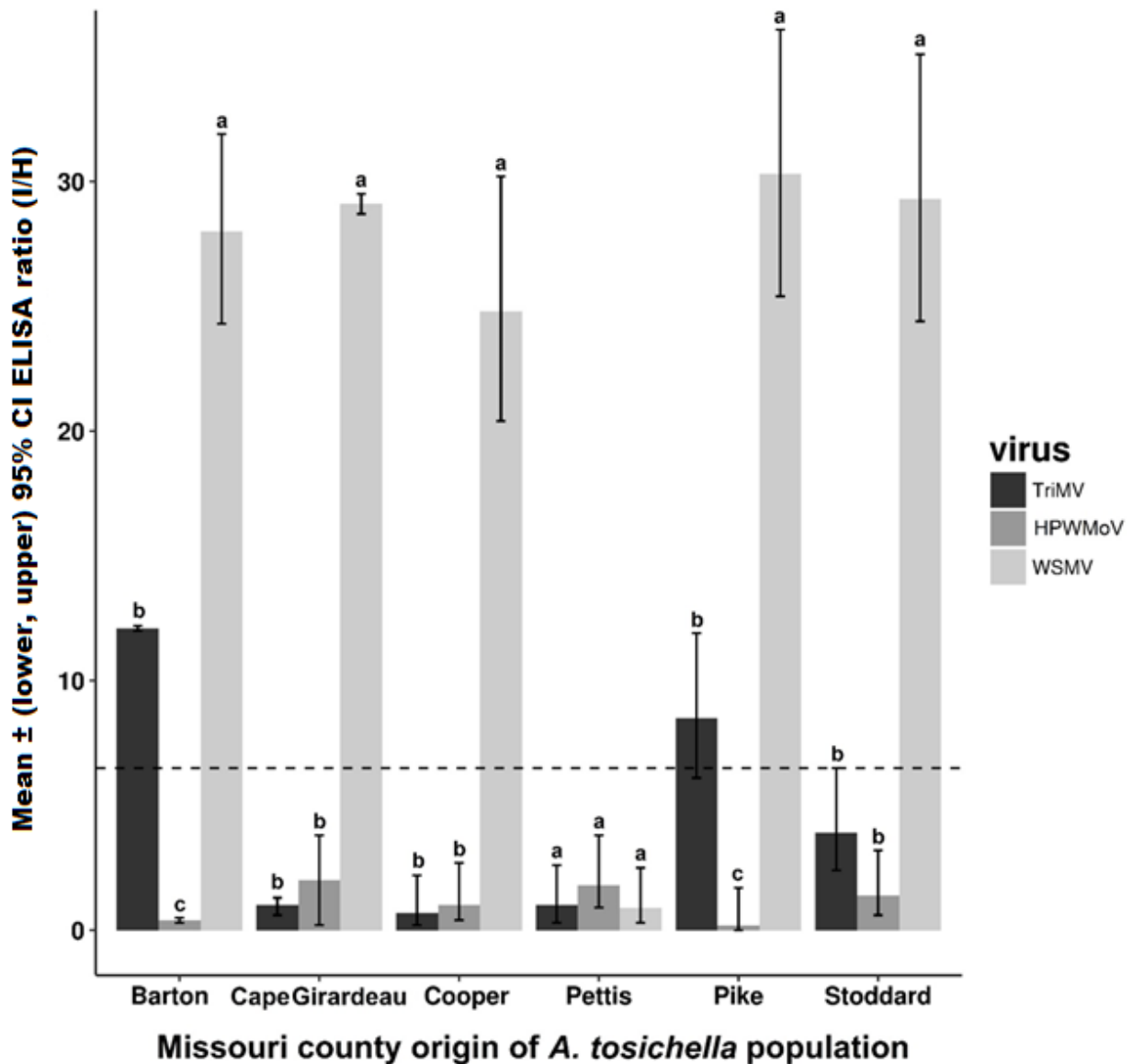


Figure 2-5. Mean \pm (lower, upper) 95% CI ELISA ratios of *Wheat Streak Mosaic Virus* (WSMV), *Wheat Mosaic Virus* (WMoV) and *Triticum Mosaic Virus* (TriMV) detected in *A. tosicHELLa* collected at six locations in Missouri after 21 d of feeding on plants of the *A. tosicHELLa*-susceptible Jagger wheat. a, b- Means followed by a different letter at each location differ significantly at $p < 0.05$ (Tukey's HSD test). ELISA ratio = (OD₄₀₅ value of infected leaf/OD₄₀₅ value of healthy uninfected leaf). Dashed line = maximum ELISA threshold ratio for virus-free control plants.

Discussion

Assessing virulence of wheat curl mites from several geographic sites over time is very important to facilitate a management strategy (Harvey et al. 1999). This is especially true, given the documented ability of mites to overcome antibiosis resistance in wheat cultivar TAM107 (Harvey et al. 1999). However, other sources of resistance have been reported that reduce mite populations (Carrera et al. 2012; Chuang et al. 2017; Aguirre-Rojas et al. 2017). Results of our experiments, to our knowledge, are the first report since those conducted by Harvey et al. (1999) to provide a robust survey of mite virulence to different wheat curl mite resistance genes and the viruses associated with different mite populations.

In general, *Cmc4* resistant control plants showed from 0-20% leaf folding, suggesting that *Cmc4* remains an effective mite resistance gene in wheat in the U. S. Great Plains (Carver et al. 2016). Plants of the KSU2R-2 breeding line containing *Cmc4* and *Wsm2* also showed significantly less leaf folding than susceptible Jagger plants in response to all mite populations except those in three counties in Kansas and three counties in Missouri (Tables 2, 3, 4). These leaf folding responses are similar to those determined by Chuang et al. (2017). Interestingly, mite populations from Greeley County Kansas; Cooper County, Missouri; and Hughes County, South Dakota, were avirulent to PI52452 (*Cmc2*) and TAM107 (*Cmc3*), suggesting that these genes remain an effective source of mite resistance in some locations (Tables 1 and 3).

Plants containing *Cmc2* displayed significantly less leaf folding in response to feeding by mites from Hughes County, South Dakota; and plants containing *Cmc3* displayed significantly less leaf folding in response to feeding by mites from Tripp County, South Dakota; and Bottineau and Ward County North Dakota. Plants containing *Cmc4* + *Wsm2* exhibited significantly less leaf folding in response to feeding by all mite populations (Table 3 and 4).

These results demonstrate that *Cmc2* and *Cmc3* resistance remains effective against some mite populations in the U.S. Great Plains.

WSMV was much more prevalent in the mite populations than either HPWMoV or TriMV, occurring in 76% of the populations sampled, compared to the occurrence of HPWMoV and TriMV at only 16% and 8% of the locations, respectively. Seifers et al. (2002, McMechan et al. 2014) reported that mite populations capable to transmit WSMV, HPWMoV, and TriMV at rates 74-100%. McMullen and Nelson (1989) reported 37% of North Dakota's fields infected with WSMV in the spring. Burrows et al. (2009, 2016) also reported a higher WSMV frequency (47%) than HPWMoV (19%), TriMV (17%), or BYDV-PAV (7%) in winter wheat plant in nine states in the U. S. Great Plains region. Rotenberg et al. (2016) reported the occurrence of BYDV-PAV and WSMV in spring at Kansas about the same 22% and 19% respectively. Oliveira-Hofman et al. (2015) suggested that WSMV predominance has persisted because it is transmitted by both wheat curl mite biotypes. However, our data sets show not only presence or absence of WSMV infection (Burrows et al. 2009; Byamukama et al. 2013), but also contain ELISA ratios that allow comparisons of virus infection magnitude in plants of genotypes with different mite resistance genes (Fahim et al. 2012). In addition, we determined that mites collected from three counties in North Dakota and South Dakota contained WSMV and HPWMoV at significantly greater levels than TriMV (Figure 3 and 4, Supplementary Table 2). Finally, our results are also similar to those reported by Burrows et al. (2009), as neither study detected more than two viruses present within any one location, and the absence of all three viruses co-occurring in mites from any location.

Wheat curl mite populations in our study were taken in each of the same eight counties in Kansas sampled by Harvey et al. (1999) in 1996 and 1997. Comparisons of mite virulence and

avirulence indicate that virulence to *Cmc2* increased or remained the same in six of the eight counties between 1997 and 2014 and diminished from virulence to intermediate (not different from resistant or susceptible control plant response) in Ellis and Finney counties. Virulence to *Cmc3* increased only in mites from Finney County, remained the same in Barton, Ellis, and Saline counties, and diminished from virulent to intermediate in mites from all other counties sampled in Kansas. Taken together, these results demonstrate that levels of wheat curl mite virulence to *Cmc3* in Kansas have diminished since 1997 and increased slightly to *Cmc2*.

Reductions in mite populations resulting from feeding on breeding line KSU2R-2 (*Cmc4* + *Wsm2*) paralleled leaf folding scores on KSU2R-2 plants. Wheat curl mite populations on KSU2R-2 plants were significantly lower than those on susceptible plants in 20 of 25 populations sampled (Table 5). In general, our results suggest that KSU2R-2 (*Cmc4* + *Wsm2*) is a strong source of mite resistance with excellent potential for use in U.S. Great Plains wheat breeding programs. This resistance has been recognized by breeders in Oklahoma and Montana, where mite-resistant cultivars containing *Cmc4* have been developed and released (Hofer et al. 2011, Carver et al. 2016). However, the intermediate levels of avirulence to *Cmc4* in four populations from Kansas, Missouri, and Nebraska reinforce the need for continued virulence monitoring in areas of chronically high wheat curl mite populations.

Acknowledgments

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Chapter 3 - Wheat curl mite biotype composition in the U.S. Great Plains

Abstract

The wheat curl mite, *Aceria tosichella* Keifer, is one of the most destructive arthropod pests of bread wheat worldwide and has a significant impact on yield reduction. Moreover, *A. tosichella* is the only vector for several economically important wheat viruses in the North American Great Plains. To date, mite-resistant wheat genotypes are the only effective method of controlling the *A. tosichella* - virus complex. Thus, it is important to elucidate the population genetic structure of *A. tosichella* since this can have a direct bearing on mite resistance management. Several previous studies have detected two genetically distinct lineages of *A. tosichella*. In this study, DNA was extracted from individual mites within each of 38 populations collected from locations in six wheat-producing states in the U. S. Great Plains. Amplification of the internal transcribed spacer 1 (ITS1) and COI regions was used to characterize *A. tosichella* biotype composition and model spatio-temporal dynamics based on biotype prevalence. Results showed that the ratio of biotype 1 and 2 varies by location. Greater ranges of cropland and grassland within 5000m of the sample site, as well as higher mean monthly precipitation during the month prior to sampling appeared to reduce the prevalence of biotype 1.

Introduction

The wheat curl mite, *Aceria tosichella* Keifer, is a global pest of bread wheat *Triticum aestivum* L. The mite plays a role in reducing yield by causing direct damage and as a vector of several viral wheat pathogens (Slykhuis 1955; Nault and Styer 1970; Harvey and Martin 1992; Amrine and Stany 1994; Harvey et al. 2000; Seifers et al. 2009; Murugan et al. 2011; Navia et al. 2013). Harvey et al. (2000, 2002) estimated the yield loss caused by *A. tosichella* itself up to 30% due to leaf rolling and trapping (Orlob 1966). *A. tosichella* transmits three damaging wheat viruses in the central U.S. and Canada – Wheat Streak Mosaic Virus (WSMV, family Potyviridae, genus *Tritimovirus*), *High Plains wheat mosaic virus* (HPWMoV, genus *Emaravirus*, formerly *High plains virus*; www.ictvonline.org/proposals-15/2015.018aP.A.v3.Emaravirus_sp.pdf), and *Triticum Mosaic Virus* (TriMV, family Potyviridae, genus *Poacevirus*).

Aceria tosichella nymphs obtain WSMV after feeding for as little as 30 min on infected plants and can spread the virus for at least 7 days postfeeding. (Slykhuis 1955; Orlob 1966; Seifers et al. 1997, 1998; Hadi 2011; Appel et al. 2015). However, WSMV has the highest incidence vis-a-vis HPWMoV and TriMV (Seifers et al. 2011). Often, multiple viruses co-infect plants within a location (Mahmood et al. 1998; Seifers et al. 2008; Byamukama et al. 2013). Navia et al. (2009) described WSMV as a major pathogen of wheat on at least five different continents. Average wheat yield losses ranging from 2.5 to 7% have been reported to result from WSMV infection. This variation has been shown to depend on climate, the time required for plants to become infected, and the wheat cultivar (Tosic 1971; Sim et al. 1988; Christian and Willis 1993; Seifers et al. 1996; Sánchez-Sánchez et al. 2001; Appel et al. 2015; Rotenberg et al. 2016).

It is difficult to determine the presence of *A. tosichella* on the plant because of the mite's small size, which ranges from 150 – 225 µm, depending on the mite developmental stage (Orlob 1966), its unique protective behavior of hiding among plant leaves, and its wide range of host plants (Slykhuis 1955; Connin 1956; Somsen and Sill 1970; Harvey et al. 2001; Amrine and de Lillo 2003; Skoracka and Kuczynski 2006; Carew et al. 2009; Navia et al. 2009; Skoracka et al. 2012, 2013). To date, no effective acaricides exist to manage *A. tosichella* and the viruses with which it is associated (Martin et al. 1984; Velandia et al. 2010). Currently, *A. tosichella*-resistant wheat cultivars are the only feasible approach to control *A. tosichella* (Wood et al. 1995; Harvey et al. 1999; Thomas et al. 2004; Murugan et al. 2011; Carrera et al. 2012). Resistant wheat cultivars slow the *A. tosichella* reproductive rate, consequently reducing the *A. tosichella* population size and WSMV infection incidence (Harvey et al. 1990, 1994, 2005; Conner et al. 1991). Andrews and Slykhuis (1956) were the first to identify mite resistance in hybrid wheat by crossing *A. elongatum* and *A. intermedium* to wheat. However, some *A. tosichella*-resistant wheat cultivars have been overcome by virulent strains of *A. tosichella* (Harvey et al. 1995, 1997, 1999).

Slykhuis (1955) was the first to demonstrate that *A. tosichella* has more than one biotype. Frost (1995) reported that there were two types of *A. tosichella* in Australia occurring on wheat. Harvey et al. (1995, 1999, 2001) and Malik et al. (2003) showed repeatedly that *A. tosichella* populations from different geographic locations in North America differ in their virulence and response to wheat resistance genes, while Seifers et al. (2002) emphasized that *A. tosichella* populations differ in their ability to transmit WSMV and HPWMoV. Skoracka and Kuczynski (2006) clarified morphological differences in *A. tosichella* populations in Poland.

U.S. biotypes 1 and 2 were initially identified by Malik (2001) using an internal

transcribed spacer 1 (ITS1) region. These results were later confirmed by Siriwetwivat (2006) and Hein et al. (2012) in the U.S.; and by Carew et al. (2009) and Schiffer et al. (2009) in Australia. Both biotypes occur together on both continents. Schiffer et al. (2009) also determined that WSMV is transmitted only by *A. tosichella* biotype 2 in Australia. Most recently, Skoracka et al. (2012, 2013) determined at least eight genetic lineages in a *A. tosichella* complex in Poland.

Although *A. tosichella* virulence has remained stable for the past 20 years (Chuang et al 2017), very little information exists about the current distribution of *A. tosichella* biotypes throughout the U. S. Great Plains and the potential occurrence of new biotypes. In order to obtain new knowledge for more effective IPM programs of *A. tosichella*, a regional study was conducted to assess the current genetic variation of *A. tosichella*. Our hypothesis was that *A. tosichella* biotypes change over time. To test this hypothesis, experiments were conducted to assess the distribution of *A. tosichella* biotypes in six U. S. Great Plains wheat-producing states in 2014 and 2015 based on internal transcribed spacer 1 (ITS1) and cytochrome oxidase I (COI) polymorphisms and plant phenotypic reactions. An additional experiment was conducted to compare in-depth sequence analyses of *A. tosichella* populations at four locations in Kansas, Missouri and Nebraska in 2016 to determine variation over local scales. Finally, temporal variation in *A. tosichella* lineages over a 2-year period was used to develop a generalized additive spatio-temporal model to predict the prevalence of biotypes I and II in the Great Plains.

Materials and Methods

***Aceria tosichella* sample collection**

Aceria tosichella was collected from wheat *T. aestivum* heads in 25 locations in the U.S. Great Plains wheat production area from May 21 to July 10, 2014; June 25 to July 12, 2015; and

June 11 to June 16, 2016. We took samples in 2016 from the same sites (as in 2014 and 2015) or the nearest wheat field. The GPS coordinates of each sample location are shown in supplementary tables 1 and 2. Three fields were sampled at each location and in each field 30 wheat heads were sampled (Schiffer et al. 2009), resulting in a total of 90 heads per location. To avoid bias, the heads were pooled and 10 heads from each location were arbitrarily selected for analysis. One individual live female was transferred under the microscope from each wheat head to a cold microcentrifuge PCR tube and centrifuged at 14,000 rpm at 4°C for 1 min to position the mite near or in the bottom of the tube before storage at -80°C. An 8 h recess was observed between transfers to prevent cross-contamination between populations (Orlob 1966). In 2016, additional collections were made at four locations in Kansas, Missouri, and Nebraska (Table 2). Three wheat fields were sampled at each location, and in each field, five heads were sampled from each of three sites, resulting in a total of 45 heads per location. Each head was kept separate in a plastic bag in order to distinguish genetic differences between mites within a field and a grain head.

***A. tosichella* DNA processing and amplification**

Aceria. tosichella DNA was extracted using the MyTaq™ Extract-PCR kit (Bioline USA Inc. Taunton, MA). A master mix was prepared for each reaction using 35 µl nuclease-free water (Ambion Co., Lewisville, TX), 10 µl Buffer A and 5 µl Buffer B (total 50 µl). This solution was added to each tube containing a *A. tosichella*. Tubes were incubated at 75°C and 95°C for 10 min each and thereafter held at 12°C for ∞. Mite DNA extracts were stored at 4°C. Polymerase chain reactions (PCRs) were performed to amplify 618 base pairs (bp) of the nuclear ribosomal internal transcribed spacer 1 (ITS1). Primer3Plus (Untergasser et al. 2007) was used to design primers to amplify 600 bp of this gene (Table 1). A subsample of specimens was subjected to cytochrome

oxidase I (COI) analysis to confirm whether biotype groupings/designations were correct. All PCRs were conducted in a 40 µl volume including 1 µl DNA extract, 20 µl Taq DNA polymerase (Bioline Inc. Taunton, MA), 0.5 pmol each of the forward and reverse primers (Table 1), 1 µl MgCl₂ (Thermo Scientific, New Hampshire, MA) and 17 µl nuclease-free water, using a T100 thermal cycler (Bio-Rad, Hercules, CA).

The ITS1 amplification protocol was 95°C for 3 min (initial denaturation), four cycles of 95°C for 20 sec, 56°C for 15 sec, 72°C for 20 sec, followed by 34 cycles of 95°C for 20 sec, 45°C for 15 sec, 72°C for 20 sec, and 72°C for 15 min. The COI amplification protocol was 95°C for 3 min (initial denaturation), 40 cycles of 95°C for 20 sec, 45°C for 15 sec, 72°C for 20 sec, and 72°C for 15 min. 5 µl of each PCR product was mixed with 1 µl loading dye (Promega, Madison, WI) and run on a 1% agarose gel (Fisher Scientific, Suwanee, GA), stained with GelGreen-® Nucleic Acid Gel Stain (Bioline Inc. Taunton, MA) for 60 min and visualized under UV light (Bio-Rad Gel Doc EZ System Gel Imaging System, San Jose, CA). PCR product sizes were assessed using the Hi-Lo™ DNA marker (Minnesota Molecular, Inc. Minneapolis, MN).

The PCR product concentration was measured by comparison with Lambda DNA of standard concentrations (Promega) and Nanodrop spectrophotometry (Thermo Scientific). PCR cleanup to remove remaining dNTPs, primers, Taq, and Mg⁺ and all sequence data were generated by GeneWiz Inc. (South Plainfield, NJ). Because of large sample sizes, PCR products were sequenced for a few specimens in both directions (F and R) using the same primers used for PCR. However, the majority of our specimens were sequenced in one direction (F) only. Sequences for *A. tosichella* and related species were aligned and edited using BioEdit V. 7 software (Hall 1999). Neighbor-joining trees with 1000 bootstrap replicates were constructed with MEGA7 software (Tamura et al. 2011) for ITS1 and COI, using a distance-based method

calculated with the Kimura-2-parameter model. In addition, sequences were imported nexus files to POPART (Leigh and Bryant 2015) to create phylogenetic network diagrams for ITS1.

Maximum likelihood phylogenetic analyses (PhyML 3.0) (Guindon et al. 2010) were conducted with the France National Institute of Bioinformatics (IFB) software, available at

<http://www.france-bioinformatique.fr/>, using the best-fit models of nucleotide substitution.

Bayesian phylogenetic analyses of the data were performed using MrBayes 3.2 (Ronquist et al. 2012). DnaSP v. 5.10.01 (Librado and Rozas 2009) was used to test polymorphism among individuals within each genes (ITS1 and COI). The nucleotide sequences of ITS1 and COI used in phylogenetic analyses have been deposited in GenBank (Accession numbers will be inserted after acceptance). A sequence of the *A. eximia* (JF920113.1) obtained from Genbank and used as an outgroup was included in analyses.

Spatio-temporal prediction of *A. tosichella* biotype

A generalized additive model was used to capture the spatio-temporal dynamics in the prevalence of *A. tosichella* biotypes 1 and 2, incorporating weather and land cover as dependent variables with temporal changes in *A. tosichella* population dynamics. A binomial distribution was assumed, with the number of “trials” of the binomial distribution being the number of mites sampled at each unique site and time period, which was 10 in 2014-2015 and 15 in 2016.

For each sample obtained, the PRISM database (PRISM 2017) was used to obtain the average monthly temperature and precipitation occurring during the month and the month prior to sample collection, and the 2011 National Land Cover Database (Homer et al. 2015) was used to determine either grass/pasture or cropland land cover covariates at the 30 m by 30 m resolution. NLCD classes 71 and 82 defined grass/pasture and class 42 defined cropland. Land cover was assumed to influence mite prevalence at a scale larger than 30 m x 30 m resolution.

The effective scale influencing the response was determined by calculating the percentage of grass/pasture and cropland within circular regions centered at the sample location with a diameter of 100-, 500-, 1000-, 2500-, 5000-, and 10000m.

Spatio-temporal effects unrelated to weather or land cover covariates i.e., autocorrelation (Hefley et al. 2017) were included using a categorical factor composed of the year of data collection and thin plate regression splines, a type of basis function that models “smooth” effects of spatial location or time (Hefley et al. 2017). The interaction between grass/pasture and cropland land cover at the 500m scale was included in a given model, but candidate models were constructed for spatial scales at 100-, 500-, 1000-, 2500-, 5000-, and 10000m. The appropriate scale was chosen from the candidate model with the lowest AIC score (Burnham and Anderson 2002) and calculating the Akaike's information criterion (AIC). The drivers of the prevalence of each *A. tosicHELLa* biotype were assumed to covariate with coefficients within 90% confidence intervals that did not contain zero.

Results

***A. tosicHELLa* biotype distribution**

ITS1 analysis was conducted on a total of 250 *A. tosicHELLa* collected in 2014 and 2015 from 25 locations in Kansas, Nebraska, Missouri, North Dakota, South Dakota, and Texas (10 mites per location) and 45 mites collected in 2016 from four locations in Kansas, Missouri and Nebraska (15 mites per location) (Supplementary Tables 3-1 and 3-2). In each mite sample, a region of 618 bases was obtained for the ITS1 gene and analyzed, and in an additional 49 samples, a region of 506 bases of the COI gene were obtained and analyzed. Sequencing results showed that all mites sampled were *A. tosicHELLa* (Carew et al. 2009 and Hein et al. 2012). Bayesian phylogenetic

analyses revealed clearly distinct differences between biotypes 1 and 2, based on 8 ITS1 haplotypes and 9 COI haplotypes.

In general, *A. tosichella* biotype 1 occurred in greater frequency in Kansas, Missouri and South Dakota (50-70%), compared to a much lower frequency (17.5-40%) in North Dakota and Nebraska, to total absence in Texas (Table 3-2). Biotypes 1 and 2 were found in all locations sampled, with the exception of both counties sampled in Texas and two counties sampled in Nebraska, where only biotype 2 was present. Out of 250 mites sequenced, biotype 1 comprised 45.6 % (114 sequences) whereas biotype 2 comprised 54.4 % (136 sequences) (Table 3-2). In Kansas, the overall ratio between biotypes 1 and 2 was 50:50. However, biotype 1 comprised as little as 20% of the total population in Ellsworth County, and as much as 90% of the total population in Geary County. In contrast, biotype 2 was prevalent in Nebraska, with the total population comprised of 82.5% biotype 2 and only 17.5% biotype 1. The lowest prevalence of biotype 2 occurred in Hayes County, Nebraska (60%), and the highest prevalence (100%) occurred in Furnas and Saunders Counties, Nebraska. In Missouri, the ratio between biotypes 1 and 2 was 70:30, with the prevalence of biotype 1 ranging from 40% in Cooper County to 90% in Cape Girardeau County. The biotype ratio was 40% biotype 1 and 60% biotype 2 in North Dakota, and 57% biotype 1 and 43% biotype 2 in South Dakota, where the prevalence of biotype 1 ranged from 20% in Lake County to 80% in Tripp County (Table 3-2).

Results of biotype ratio determinations between 2014 and 2015 are shown in (Table 3-2) and 2016 shown in (Table 3-3). In general, the percentage of biotype 1 decreased in Ellis County, Kansas; and Barton and Cape Girardeau counties, Missouri. In Ellis County, the percentage of biotype 1 decreased from 70% in 2014, to 42% in 2016. In Cape Girardeau County, the percentage of biotype 1 decreased from 90% in 2015, to 76% in 2016 and in Barton

County, the percentage of biotype 1 decreased from 80% in 2015, to 60% in 2016. In Hayes County, Nebraska, the percentage of biotype 1 increased from 40% in 2015, to 55% in 2016.

Calculations of genetic distance and genetic identity between all *A. tosichella* populations based on ITS1 indicated that distances ranged from 0.003 between Barton and Cape Girardeau Counties, Missouri, to 0.028 between Dallam County, Texas and Barton County, Missouri. Genetic similarity identity ranged from 93% between Barton County, Missouri and Dickinson County, Kansas to 100% between Dickinson- and Ellis Counties, Kansas within *A. tosichella* biotype 1 (Table 3-4). Genetic distance and genetic identity values based on COI variation ranged from 0.002 to 0.204 while genetic similarity ranged from 85- to 100% (Table 3-5).

Phylogenetic network diagrams revealed two *A. tosichella* biotypes within the Great Plains in 2014, 2015, with the exception of Texas, where only biotype 2 occurred, and that there is higher variation in biotype 1 than biotype 2 (Fig. 3-1A). Five haplotypes were found to belong to *A. tosichella* biotype 1 and three haplotypes belonged to *A. tosichella* biotype 2. A haplotype of *A. tosichella* biotype 1 with a 1bp difference to the primary haplotype was found in about one third of the mite populations and was present in four states, primarily in Kansas and Missouri. *A. tosichella* biotype 2 was present in six states. Most of the variation in this biotype was observed in Kansas and Texas (Fig. 3-1A). Samples collected in 2016 also showed two distinct biotypes present in all locations sampled as in 2014-2015. Interestingly, a new haplotype within biotype 1, that differed by 1bp to the dominant haplotype, appeared in 2014, 2015, and 2016 (Fig. 3-1 A and B).

Local, regional and temporal variation in biotype ratios

Biotype composition differed within regions (Table 3-2) as well as within fields (Table 3-3). In general, both biotypes were present in a field in varying ratios. Exceptions to these were

eight fields, six of them in Cape Girardeau County, Missouri, where only biotype 2 was present. The biotype 1 composition varied from 20-60% in Ellis County, Kansas; to 40- 80% in Barton County, Missouri; to 0-100% in Cape Girardeau county, which was where most of the variation occurred; and from 40-100% in Hayes County, Nebraska (Table 3). Interestingly, the percentage of biotype 1 was higher than biotype 2 in Hayes County, Nebraska; Barton and Cape Girardeau County, Missouri, whereas the situation was reversed in Kansas, with biotype 2 occurring at a higher frequency than biotype 1.

The results from 2016 samples based on individual wheat heads indicated that both biotypes could occur in a head simultaneously. Three heads were examined from each of 12 fields, for a total of 36 heads. Out of these, 28 heads contained both biotypes (Fig. 3-2). Of the eight heads that contained a single biotype, six were collected in Cape Girardeau, Missouri (Fig. 3-3A, B). In a single instance, only one biotype was found in each of three heads collected from the same field (Fig. 3-3C). Only biotype 1 individuals were found in a single head collected in both Barton (Missouri) and Hayes (Nebraska) (Table 4).

Prediction of the prevalence of biotypes 1 and 2

The spatio-temporal dynamics in the prevalence (the occurrence probability) of biotypes 1 and 2 showed distinct spatio-temporal patterns (Fig. 3-4). The 5000m land cover covariate scale captured the spatio-temporal dynamics necessary to predict the prevalence of biotype 1 and 2. The percentage of grass/pasture and crops within a 5000m had negative coefficients estimates with 90% confidence intervals (CI) containing zero, indicating that higher amounts of cropland and grassland may reduce the prevalence of biotype 1. The mean monthly precipitation during the month prior to sampling was the only weather covariate with a 90% CI for a coefficient

estimate containing zero. This estimate was also negative, indicating that higher precipitation may reduce the prevalence of biotype 1 and consequently, increase the prevalence of biotype 2.

Substantial shifts in *A. tosicella* biotype abundance over time revealed a mixture of both biotypes in Kansas, Missouri, Nebraska, South Dakota, North Dakota and Texas. Biotype 1 prevalence ranged from 20-90% and biotype 2 prevalence ranged from 10-80% depending on the location and time (Fig. 3-5, 6, 7, 8, 9, 10).

Table 3-1. Primers used to amplify nuclear ribosomal internal transcribed spacer one (ITS1) and cytochrome oxidase I (COI) in *A. tosichella* biotype 1 and 2

Region	Primer name	Sequence	Reference
rDNA –	WCM_ITS1_A_F	5'-GTG AGG CAT CTG GAC TTG CT-3'	This study
ITS1	WCM_ITS1_A_R	5'-TTG TTT GCA CGC AGT CAT GG-3'	This study
	WCM_ITS1_B_F	5'-ATC CTT CAT CAC GAC TCG GC-3'	This study
	WCM_ITS1_B_R	5'-CCC TCA TAC AGG CAA GGC TC-3'	This study
mtDNA - COI	1718 F	5' -TATAAACYTCDGGATGNCCAAAAAA-3'	Simon et al. 1994
	bcdR04	5'TATAAACYTCDGGATGNCCAAAAAA-3'	Skoracka and Dabert 2010

Table 3-2. Ratios of *A. tosichella* biotype 1 and 2 in Kansas, Missouri, Nebraska, North Dakota, South Dakota and Texas in 2014 and 2015. n = 10 in each county population.

State	County	Biotype 1	Biotype 2	% Biotype 1	% Biotype 2
KS (n=80)	Saline	6	4	60	40
	Geary	9	1	90	10
	Greeley	4	6	40	60
	Dickinson	3	7	30	70
	Barton	5	5	50	50
	Finney	4	6	40	60
	Ellis	7	3	70	30
	Ellsworth	2	8	20	80
	Average	5	5	50	50
NE (n=40)	Cheyenne	3	7	30	70
	Hayes	4	6	40	60
	Furnas	0	10	00	100
	Saunders	0	10	00	100
	Average	1.75	8.25	17.5	82.5
MO (n=60)	Barton	8	2	80	20
	Cape Girardeau	9	1	90	10
	Pike	7	3	70	30
	Pettis	8	2	80	20
	Stoddard	6	4	60	40
	Cooper	4	6	40	60
	Average	7	3	70	30
ND (n=20)	Ward	5	5	50	50
	Bottineau	3	7	30	70
	Average	4	6	40	60
SD (n=30)	Hughes	7	3	70	30
	Tripp	8	2	80	20
	Lake	2	8	20	80
	Average	5	5	50	50
TX (n=20)	Randall	0	10	0	100
	Dallam	0	10	0	100
	Average	0	10	0	100

Table 3-3. Ratios of *A. tosichella* biotype 1 and 2 in Kansas, Missouri and Nebraska in 2016. A total of 3 fields in each state (12 fields total) were sampled, 5 individuals collected at each of 3 sites in each field (15 total individuals /field).

State	County	Field (n=5)	Biotype 1 %	Biotype 2 %	Average of Type 1 %	Average of Type 2 %
KS	Ellis	1.1	60	40	42	58
		1.2	20	80		
		1.3	60	40		
		2.1	60	40		
		2.2	60	40		
		2.3	20	80		
		3.1	20	80		
		3.2	20	80		
		3.3	60	40		
MO	Barton	1.1	60	40	60	40
		1.2	80	20		
		1.3	60	40		
		2.1	40	60		
		2.2	60	40		
		2.3	40	60		
		3.1	40	60		
		3.2	100	0		
		3.3	60	40		
	Cape Girardeau	1.1	80	20	76	24
		1.2	100	0		
		1.3	100	0		
		2.1	40	60		
		2.2	60	40		
		2.3	100	0		
		3.1	100	0		
		3.2	0	100		
		3.3	100	0		
NE	Hayes	1.1	80	20	55	45
		1.2	40	60		
		1.3	60	40		
		2.1	40	60		
		2.2	100	0		
		2.3	20	80		
		3.1	60	40		
		3.2	60	40		
		3.3	40	60		

Table 3-4. Genetic distance and genetic identity indices for eight U.S. *A. tosichella* populations, estimated using variation in unique ITS1 haplotypes.

	8-3-1	8-4-1	17-1-1	17-10-1	18-1-1	5-1-1	7-1-1	27-4-1	<i>A. eximia</i>
BT1_8-3-1/ Ellis .KS population	-	99	99	98	99	96	97	97	81
BT1_8-4-1/ Ellis .KS population	0.005	-	99	99	100	97	98	97	81
BT1_17-1-1/ Barton.MO population	0.008	0.003	-	98	99	97	97	97	81
BT1_17-10-1 Barton.MO population	0.016	0.013	0.015	-	96	93	95	95	79
BT1_18-1-1/ Cape Girardeau .MO population	0.008	0.003	0.006	0.016	-	97	97	97	81
BT2_5-1-1/ Dic.KS population	0.010	0.008	0.008	0.020	0.011	-	100	99	81
BT2_7-1-1/ Finney.KS	0.011	0.010	0.013	0.023	0.013	0.002	-	98	81
BT2_27-4-1/ Texas population	0.016	0.015	0.018	0.028	0.018	0.007	0.018	-	81
JF920113.1-2_Aceria_eximia	0.062	0.056	0.060	0.071	0.060	0.065	0.077	0.086	-

*Data above the diagonal represent the genetic similarity%, data below the diagonal represent the genetic distance.

Table 3-5. Genetic distance and genetic identity indices for eight U.S. *A. tosichella* populations estimated using variation in unique COI haplotypes.

	1-	2-	3-	4-	5-	6-	7-	8-	9-	10-
1-										
NE.Hayes2016.Field3.1C .COI	-	100	99	85	85	86	85	85	85	79
2-MO.PIKE2015.E.COI	0.004	-	100	85	85	86	86	86	86	79
3-										
NE.Hayes2016.Field2.2A .COI	0.006	0.002	-	85	85	85	85	86	85	79
4-										
NE.FURNAS2015.H.CO I	0.204	0.198	0.201	-	99	99	99	100	99	92
5-										
MO.BARTON2015.I.CO I	0.198	0.198	0.201	0.008	-	99	99	100	99	91
6-										
KS.EIIS2016.FIELD2.2 D.COI	0.196	0.196	0.198	0.006	0.006	-	100	100	100	92
7-										
KS.EIIS2016.FIELD2.3 A.COI	0.201	0.196	0.198	0.006	0.006	0.004	-	100	100	92
8-										
KS.ELLIS2016.FIELD3. 3A.COI	0.198	0.193	0.196	0.004	0.004	0.002	0.002	-	100	92
9-										
NE.HAYES2016.FIELD 3.3A.COI	0.201	0.196	0.198	0.006	0.006	0.004	0.004	0.002	-	92
10-GBCH12061- 13 <i>Aceria_tosichella</i> COI -5P JF920076	0.216	0.212	0.212	0.027	0.029	0.027	0.027	0.027	0.027	-

*Data above the diagonal represent the genetic similarity%, data below the diagonal represent the genetic distance. *Aceria tosichella* JF920076 a sequence you obtained from GenBank.

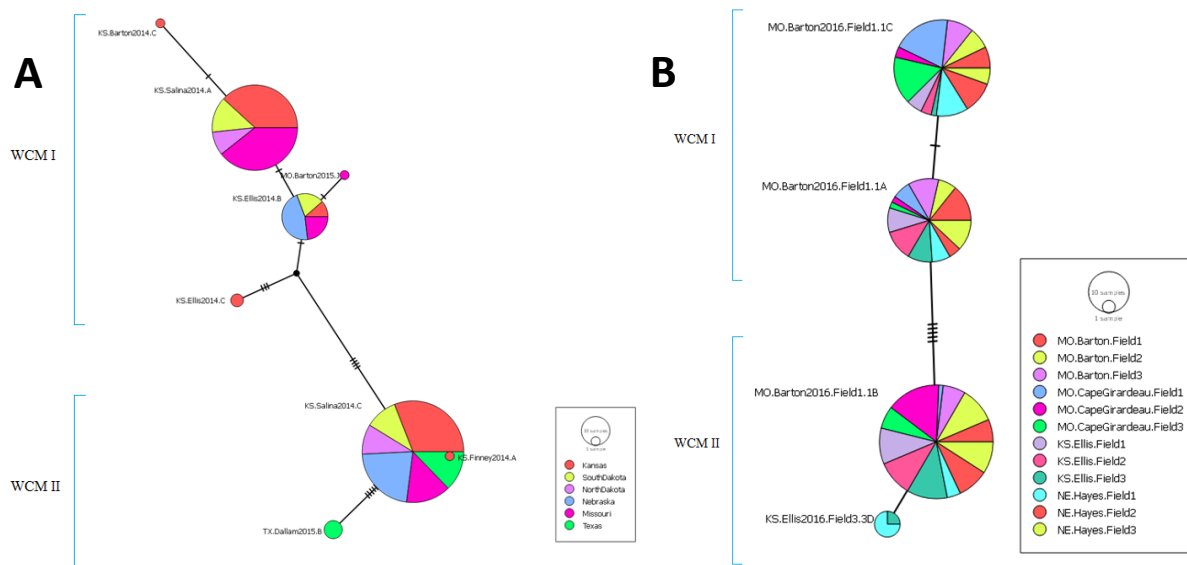


Figure 3-1. Phylogenetic network diagrams of *A. tosichella* sampled in 2014 and 2015 (A) and 2016 (B) delineating haplotypes within biotypes 1 and 2, and the size of the haplotypes of each created using PopART (Leigh and Bryant 2015). Circles symbolize haplotypes, smaller circles indicate fewer individuals in a group (haplotype). Hash marks on lines connecting haplotypes symbolize base-pair differences.

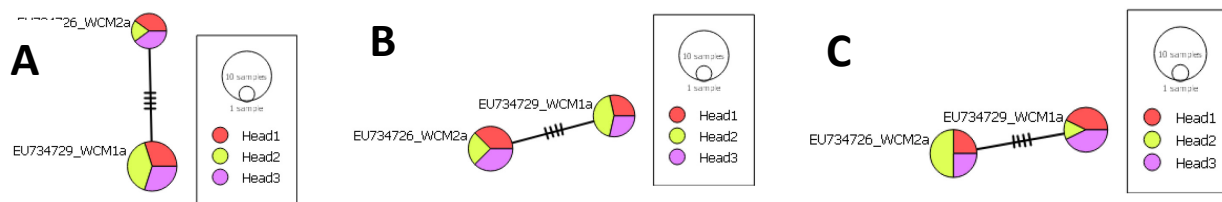


Figure 3-2. Phylogenetic network diagrams of *A. tosichella* sampled in 2016. (A) Barton county Missouri field 1 and (B) field 2; and (C) Ellis County Kansas field 1. All three fields contained biotype 1 and biotype 2 in all heads. Circles symbolize haplotypes, smaller circles indicate fewer individuals in a group (haplotype). Hash marks on lines connecting haplotypes symbolize base-pair differences. Program PopART was used (Leigh and Bryant 2015).



Figure 3-3. Phylogenetic network diagrams of *A. tosichella* sampled in 2016. (A) Barton county Missouri field 1 containing biotype 1 in all heads and biotype 2 in one head only; (B) Cape Girardeau county Missouri field 2 containing biotype 1 in heads 2 and 3 and biotype 2 all heads; (C) Cape Girardeau county Missouri field 3 containing biotype 1 in heads 2 and 3 and biotype 2 in head 2 only. Circles symbolize haplotypes, smaller circles indicate fewer individuals in a group (haplotype). Hash marks on lines connecting haplotypes symbolize base-pair differences. Program PopART was used (Leigh and Bryant 2015).

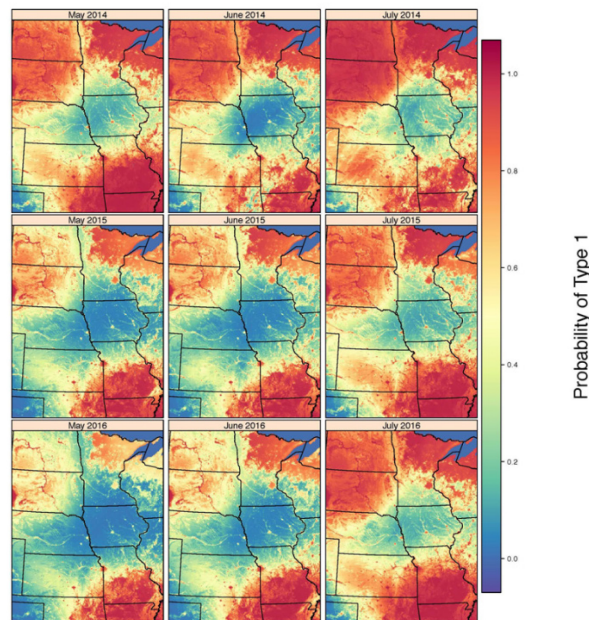


Figure 3-4. Heatmap showing the prevalence of *A. tosichella* biotypes 1 and 2 varying over space and time due to weather and land cover covariates. Areas in red have a higher probability of being biotype 1, areas in blue have a higher probability of being biotype 2.

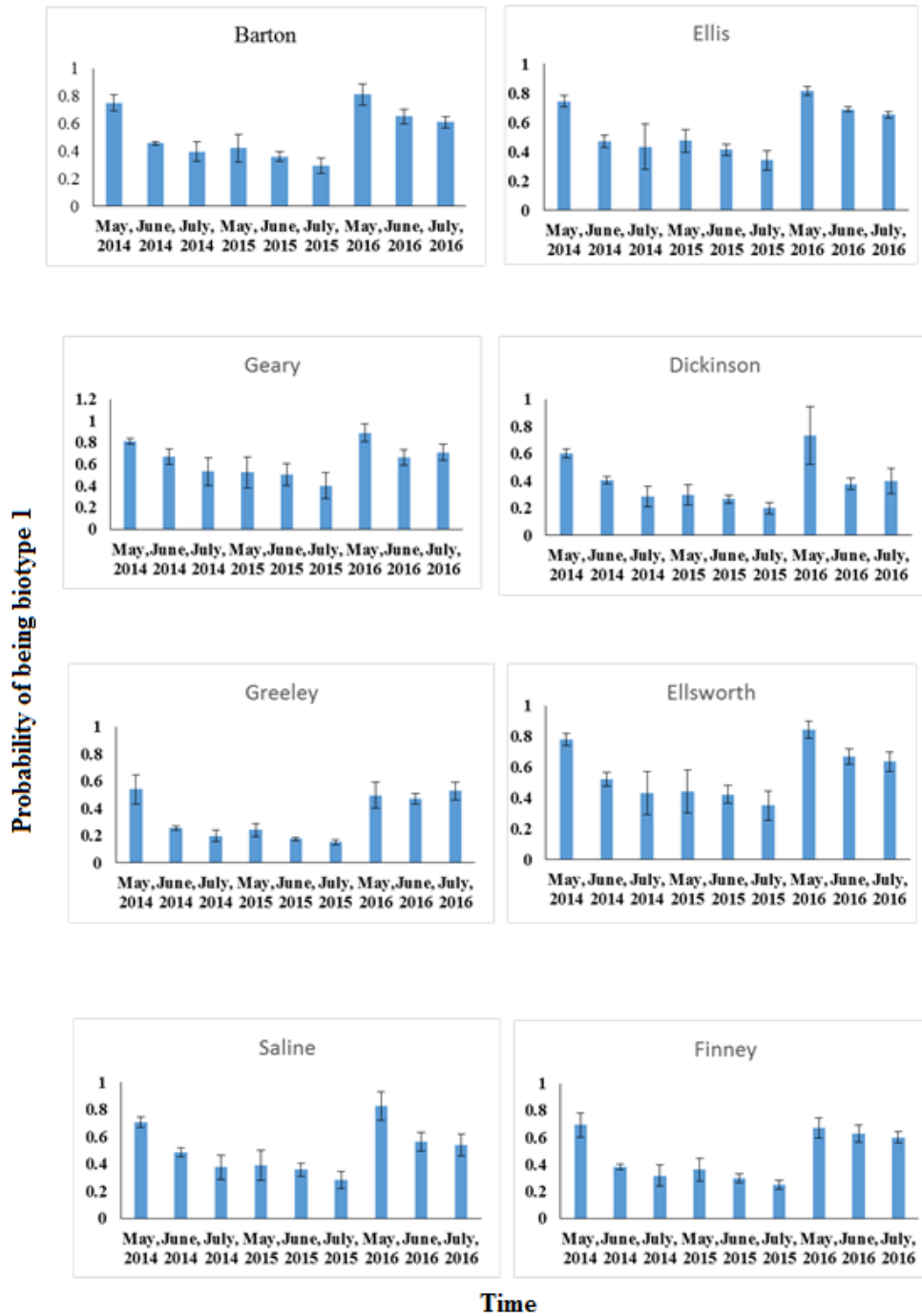
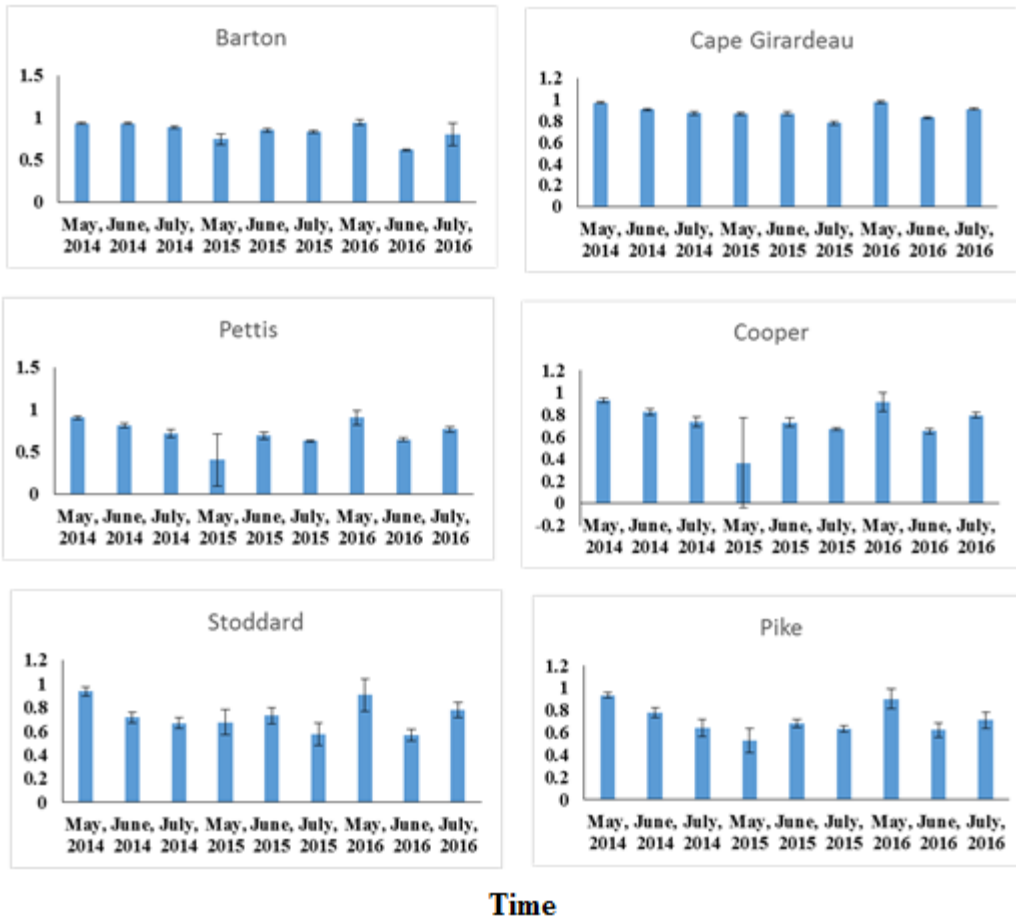


Figure 3-5. Percent of *A. tosichella* biotype 1 in eight Kansas counties.

Probability of being biotype 1



Time

Figure 3-6. Percent of *A. tosichella* biotype 1 in six Missouri counties.

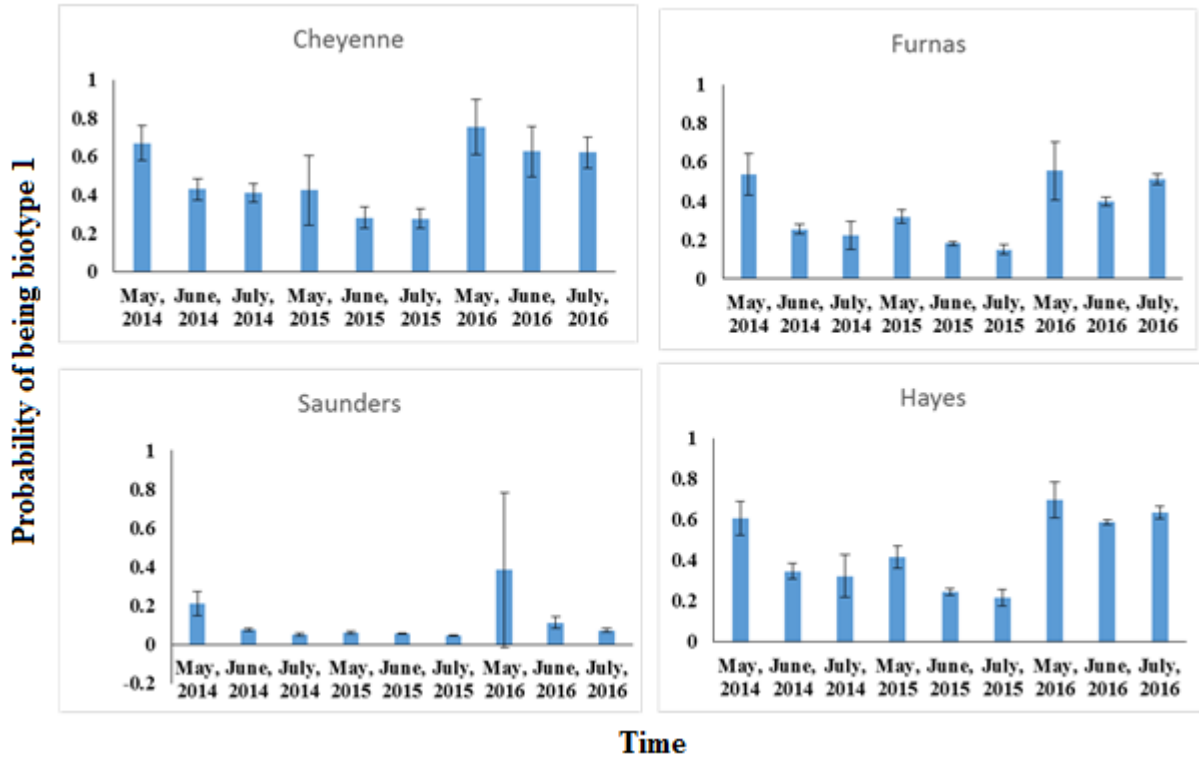


Figure 3-7. Percent of *A. tosichella* biotype 1 in four Nebraska counties.

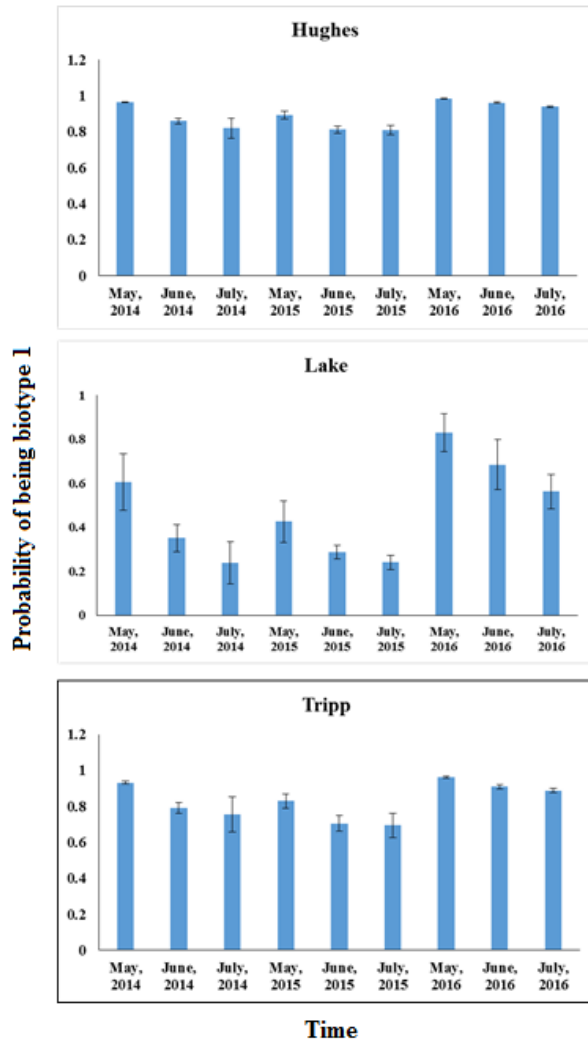


Figure 3-8. Percent of *A. tosichella* biotype 1 in three South Dakota counties.

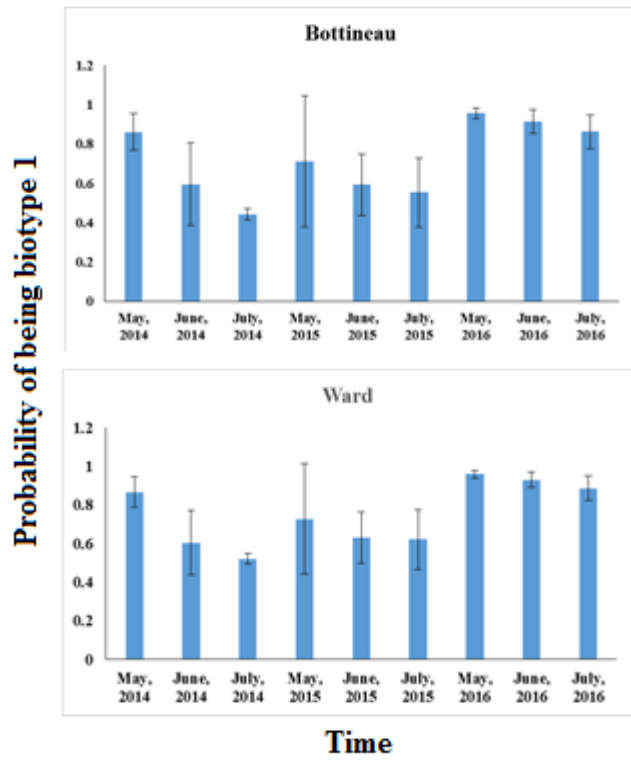


Figure 3-9. Percent of *A. tosichella* biotype 1 in two North Dakota counties.

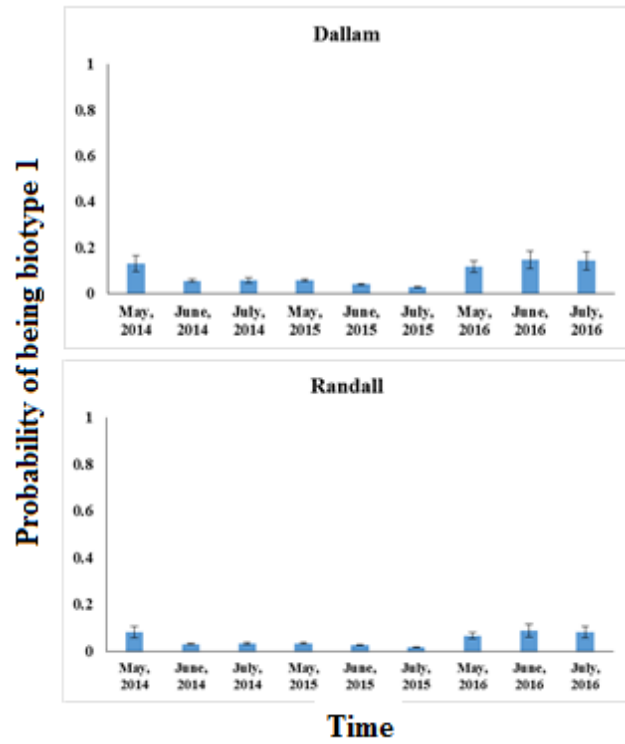


Figure 3-10. Percent of *A. tosichella* biotype 1 in two Texas counties.

Discussion

A. tosichella and the associated viruses that it transmits have historically caused major wheat yield reductions in North American wheat production. The major aims of this study were to assess the distribution of *A. tosichella* biotypes in the U. S. Great Plains based on ITS1 and COI polymorphisms and plant phenotypic reactions; and to use temporal variation in *A. tosichella* lineages to develop a spatio-temporal model predictive of biotype prevalence.

Sequencing of ITS1 and COI gene polymorphisms in the *A. tosichella* samples showed that these remain useful genomic regions for *A. tosichella* biotype discrimination (Malik 2001; Hebert et al. 2003). Both genes used in analysis support previous conclusions that *A. tosichella* has two distinct biotypes (Malik 2001; Siriwetwivat 2006; Carew et al. 2009; Schiffer et al. 2009; Hein et al. 2012; Skoracka et al. 2012, 2013). The sequence data revealed that U. S. *A. tosichella* populations with divergence in the ITS1 region were similar to the Australian haplotypes EU734729.1 (WCM1) and EU734726.1 (WCM2) (Carew et al. 2009). The results of the current study also identified 8 new haplotypes of *A. tosichella* from wheat in the populations sampled that do not match any data published in the GenBank database. The results of the current study also validated those of Streetwear (2006) showing biotype 1 and 2 co-occurrence on individual wheat heads, and those of Harvey et al. (1999) and Hein et al. (2012) showing biotype 1 predominance. Similarly, Australian biotypes 1 and 2 co-occur across Australian wheat production areas with biotype 1 occurring more often in the southeast and biotype 2 occurring more frequently in the west (Carew et al. 2009, Schiffer et al. 2009).

ITS1 is one of the best molecular regions to test for genetic variation within and between populations of mites, including *A. tosichella*, as shown by numerous previous studies (Carew et al. 2009, Hein et al. 2012, Skoracka et al. 2014). Our results further confirm the utility of the

ITS1 marker, and show that U. S. *A. tosichella* populations display genetic variation that suggest genetic drift or a host shift (Harrison 1991), although the variation is less than that in Turkey (Szydło et al. 2015), suggesting the U. S. populations could be a consequence of a genetic drift by an invasive *A. tosichella* population (Tsutsui et al. 2000; de Barro and Ahmed 2011). Alternatively, an *A. tosichella* host shift could also be the result of adaptation to mite resistance genes in wheat (Smith 2005).

Analyses of genetic distances (Table 3-4, 3-5) and phylogenetic network diagrams (Figs. 3-2, 3, 4) support conclusions of previous studies using the ITS1 and COI genes that *A. tosichella* consists of two biotypes (Malik 2001, Carew et al. 2009, Hein et al. 2012). In addition, our results indicate that differences in COI sequence variants provide greater divergence (8 haplotypes) within *A. tosichella* populations than ITS1 variation, similar to results of Hein et al. (2012). However, our results also revealed 8 haplotypes based on ITS1 sequence variants, while Hein et al. (2012) determined only two haplotypes. These differences in results are due likely to the wider geographic scope of sampling performed in our experiments (25 sample sites versus 5 sites).

In addition, our results are based on *A. tosichella* samples obtained from 2014 to 2016, while those used by Hein et al. (2012) were obtained in 1999. Finally, differences in the results of the two studies may have resulted from greater biotype diversity, resulting from release of cultivars containing the *Cmc4* resistance gene in Montana and Oklahoma (Hofer et al. 2011, Carver et al. 2016), as well as cultivation of cultivars in Colorado, Kansas, Oklahoma, and Texas with the *Dn7* gene for resistance the Russian wheat aphid, *Diuraphis noxia* (Kudjumov), and the *H21* gene for resistance to Hessian fly, *Mayetiola destructor* Say, both of which have recently been shown to be resistant to *A. tosichella* (Aguirre-Rojas et al. 2017).

Phylogenetic network diagrams in Figs. 3-1, 3-2, 3-3 provided additional evidence of two *A. tosichella* biotypes. Interestingly, ITS1 variation distinguished four different unique ITS1 haplotypes within biotype 1, each with 1 bp difference to the others. ITS1 variation in *A. tosichella* biotype 2 was less than in biotype 1, supporting conclusions of Harvey et al. (1997) that biotype 2 developed after deployment of the *Cmc3* resistance gene in wheat cultivar TAM 107 in 1983 (Martin et al. 1983).

Several attempts have been made to characterize environmental conditions that impact the prevalence of *A. tosichella* biotypes on different host plants or as virus vectors (Kuczyński et al. 2016, Skoracka et al. 2017). The generalized additive models used in the current experiments to capture the spatio-temporal dynamics of *A. tosichella* biotypes incorporated weather and land cover covariates and a distribution for the variables that matched the characteristics of each response (Hefley et al. 2017, Wood 2017). The nonlinear effects of spatial location were incorporated as well, and the model assumed a binomial distribution, where the number of “trials” of the distribution was the number of *A. tosichella* sampled at each unique site in each sample year (10 in 2014-2015, 15 in 2016). As a result, our model results provided accurate indications that precipitation and land cover affect the population dynamics of both *A. tosichella* biotypes 1 and 2 over time.

Temporal variation revealed the presence of each biotype in all one county of Kansas and Nebraska, and two counties of Missouri and in 2016 (Fig. 3-1B), with biotype 1 presence decreasing relative to biotype 2 at three of these four sites (Table 3-4). These results suggest that biotype prevalence may be related to increased spring precipitation in 2014-2016 (Parmesan 2006, Zerebecki and Sorte 2011) and/or to the fact that biotype 1 occupies plants before biotype

2 and as a result, is more prevalent (Harvey et al. 1997, 1999). However, biotype 2 is a better vector of WSMV than biotype 1 (Seifers et al. 2002, Schiffer et al. 2009, Wosula et al. 2016) and WSMV enhances biotype 2 reproduction (Murugan et al. 2011) at temperatures below ~31 °C (Kuczyńsk et al. 2016). Thus, the density of biotype 2 is likely to increase and the density of biotype 1 likely to decrease toward the end of spring. Conversely, biotype 1 prefers a temperature of ~35 °C, and its density increases in the summer (Kuczyńsk et al. 2016).

Enders et al. (2018) used similar models to determine the effects of weather and land cover on the population dynamics of different cereal aphid virus vectors. Adoption of such modeling on an area-wide basis in North America could provide additional enhanced understanding of *A. tosichella* biotype distribution. This type of information will help to improve predictions of future risk of *A. tosichella* infestations and facilitate the management of both *A. tosichella* and the viruses they transmit.

Our results provide the first accurate update to the distribution of *A. tosichella* biotypes in the U.S. Great Plains since 1997 and demonstrate biotype variation within- and between wheat fields, and within the head of the same plant. These results, plus those that demonstrate the effects of precipitation and land cover on biotype distribution, suggest the need for comprehensive, coordinated studies of *A. tosichella* biotype variation on at least a U. S. national basis, if not a global basis.

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Chapter 4 - Resistance to Wheat Curl Mite in Advanced Wheat Genotypes

Abstract

The wheat curl mite, *Aceria tosichella* (Keifer), and the viruses associated with the mite, reduce wheat yields in every wheat-producing continent. Plant resistance to mite feeding injury is a proven measure that can reduce *A. tosichella* populations. In addition, plant resistance is economically beneficial and ecologically safe. This study evaluated eight advanced wheat breeding lines for resistance to populations of *A. tosichella* biotype 1 and 2 compared to susceptible (Jagger) and resistant (OK05312) plants. The results showed that none of the advanced breeding lines were resistant to *A. tosichella* biotype 1. However, breeding lines AYN3-37 and AYN3-34 sustained significantly less damage from biotype 2 feeding than susceptible control plants and the damage was not statistically different from damage sustained by resistant plants. AYN2-28 and AYN2-36 sustained moderate resistance to biotype 2. These four advanced breeding lines could be used as sources to improve commercial varieties and reduce wheat yield losses from *A. tosichella*.

Key words: wheat, wheat curl mite, *Aceria tosichella*, arthropod-plant interaction, resistance

Introduction

Wheat, *Triticum aestivum* L, is the most important cereal grain crop in the world, and supplies 25% of proteins and 20% of the calories required by humans to one-third of the world population (Dixon et al. 2009). Despite all efforts, wheat yields in most countries are reduced by ~20% per year from arthropod pest feeding and infection by arthropod-transmitted viruses (Oerke 2006). Many of these losses occur from feeding damage by large infestations of the wheat curl mite, *Aceria tosichella* (Keifer), which damages wheat when it transmits Wheat Streak Mosaic Virus (WSMV, family *Potyviridae*, genus *Tritimovirus*), *High Plains Wheat Mosaic Virus* (HPWMoV, genus *Emaravirus*, formerly *High Plain Virus*; www.ictvonline.org/proposals-15/2015.018aP.A.v3. *Emaravirus* _sp.pdf), and *Triticum Mosaic Virus* (TriMV, family *Potyviridae*, genus *Poacevirus*). (Slykhuis 1955; Atkinson and Grant 1967; Velandia et al. 2010). Aggregate yield losses from single, double or triple viral coinfections are prevalent in the central U. S. (Burrows et al. 2009; Byamukama et al. 2012, 2013, 2014; Mahmood et al. 1998; Seifers et al. 2011). Losses from WSMV infection alone vary from 3 to 30%, depending on weather conditions and wheat cultivar (Harvey et al. 2002; Appel et al. 2015; Rotenberg et al. 2016). Controlling volunteer wheat to manage *A. tosichella* population outbreaks is complicated by several factors. Many species of range grasses serve as hosts to *A. tosichella* (Skoracka et al. 2012; Velandia et al. 2010). Delaying wheat planting until volunteer wheat is destroyed is difficult because planting dates are normally determined by availability of soil moisture, other wheat pests, and delayed planting is not feasible for growers using wheat for winter forage (Martin et al. 1984; Velandia et al. 2010). Finally, no acaracides exist for *A. tosichella* management (Morgan et al. 2005; McMechan and Hein 2016).

Thus, after nearly 150 years, wheat plant resistance remains the most economically viable and environmentally safe approach to reduce yield losses from arthropod pest infestations (Smith 1999, 2005). Wheat genotypes with heritable resistance to *A. tosichella* have existed for several decades (Andrews and Slykhuis 1956), and numerous cultivars that suppress *A. tosichella* population development have been developed and cultivated (Harvey and Martin 1988; Conner et al. 1991; Harvey et al. 1994, 2005; Chuang et al. 2017; Aguirre-Rojas et al. 2017). The goal of this study was to assess 88 breeding lines in the 2016 Kansas State University Wheat Elite Line Nursery for resistance to *A. tosichella*. Cultivars with *A. tosichella* resistance, can provide researchers additional durable sources of resistance to the mite that reduce wheat yield losses and indirectly losses due to mite-transmitted viruses.

Materials and Methods

Plant and Arthropod Material

A. tosichella biotypes 1 and 2 were maintained on the susceptible wheat cultivar “Jagger” planted in SunGrow METRO-MIX 360 potting mix (Hummert International, Topeka, KS USA). Biotype 1 originated from a field collection in Hughes County, South Dakota, and biotype 2 originated from a field collection in Cheyenne County, Nebraska, both collected in 2014. Each colony was kept in separate greenhouse rooms to prevent cross contamination between biotypes in 45 x 45 x 75 cm cages covered with 36 µm mesh screen (BioQuip, Rancho Dominguez, CA, USA). Colonies were maintained at 24:20°C day/night and a 14:10 [L: D] h photoperiod. A nuclear ribosomal internal transcribed spacer 1 (ITS1) marker (Malik 2001) was used to verify that each colony was free of contamination of other mite biotypes a week before each experiment.

Ten plants of each of the 88 genotypes in the 2016 Kansas State University Advanced Wheat Breeding Line Nursery were screened in preliminary separate greenhouse evaluations, where they were separately subjected to heavy infestations of each biotype in two separate experiments. Eight of these lines exhibited no leaf folding after 14 d of infestation in the preliminary evaluation (Table 4-1). These eight lines were then assessed for antibiosis to both biotype 1 and 2 in the greenhouse at the same environment as stated above. One seed of each of the eight breeding lines, the susceptible cultivar Jagger, and the resistant cultivar OK05312 (Carver et al. 2016) were grown in 5 x 5 x 5 cm plastic pots (Hummert International, Topeka, KS USA). All pots were then placed inside a 90 x 90 x 180 cm cage to prevent mite biotype contamination. Ten plants of each genotype in the two-leaf- stage were then infested with a piece of wheat leaf holding 10 adult mites and caged for 14 d. Separate experiments were conducted for each biotype. After assessment for leaf folding (Chung et al. 2017) all plants were cut just above the soil level, and their leaves spread on adhesive 5 x 9 cm gridded blue paper sheets. Each sheet was then stored in a 50 ml Falcon tube (Fisher Scientific, Waltham, MA USA) for 4-5 d or until the leaves dried. All tubes were kept at room temperature (Murugan et al. 2011) and placed in a tube holder at a 45° angle to prevent mites from falling into the bottom of the tube. Mites migrated from leaves to adhesive and were counted using a Nikon SMZ-645 stereo zoom microscope at 50X magnification.

Data Analyses

A. tosichella response variables for leaf folding and mite virulence were independently analyzed. Because of small sample sizes, leaf folding data were analyzed by comparing each of the eight genotypes to resistant and susceptible control cultivars using the χ^2 Fisher's Exact Test (Fisher 1954). Mite virulence experiments was arranged in completely randomized designs and data

subjected to ANOVA using PROC GLIMMIX SAS software v.9.4 (SAS 2008). A Poisson distribution was used in the analyses to account for skewness of the data. Over dispersion was assessed based on a maximum-likelihood Pearson χ^2 degrees of freedom statistic (Stroup 2015). Confidence intervals were used instead of standard errors as data did not follow assumptions of normality and homogeneity of variances according to the Shapiro-Wilk test of normality (Shapiro and Francia 1972). Means were separated by Tukey's HSD (honestly significant difference) procedure if the type III test for fixed effect was significant at $P < 0.05$.

Results

***A. tosicHELLa* leaf folding and mite virulence**

The percentage of plants with folded leaves was significantly different between the advanced breeding lines and the resistant control (Pearson $\chi^2 = 21.3$; $df = 9$; $p < 0.0088$). The mean *A. tosicHELLa* - induced leaf folding was significantly greater on the advanced breeding line plants (50-90%) compared to the resistant OK05312 control (0%) in the biotype 1 experiment (Table 4-2). There were significant differences in the mean number of *A. tosicHELLa* produced after 14 d of infestation between susceptible and resistant control plants. However, there were no statistical differences in the mean number of mites produced on the advanced breeding lines and the susceptible control ($F = 9.21$; $df = 9$; $p < 0.0001$) (Table 4-2).

Even though the percentage of leaf folding by biotype 2 varied from 0 to 60%, there were no significant differences between any of the advanced breeding lines and resistant OK05312 control plants (Pearson $\chi^2 = 17.3$; $df = 9$; $p < 0.046$) (Table 4-3). There were also significantly fewer biotype 2's on plants of AYN3-37, AYN3-34 and OK05312 than Jagger at 14 d post-infestation. Further, the AYN2-28 and AYN2-36 advanced breeding lines had biotype 2

populations intermediately resistant to but significantly less than populations on susceptible Jagger plants ($F = 5.41$; $df = 9$; $p < 0.001$) (Table 4-3).

Table 4-1. Eight advanced wheat breeding lines with putative resistance to feeding damage by *A. tosichella* biotypes 1 and 2 (no leaf folding, 14 d post infestation).

Line name	Pedigree
KS10DH0068-9	Clara CL/KS030792K-2
KS080426-M-7	KS030010~3/KS020363WM~1
KS080655-M-2	KS990160-4~5/KS020045-8//KS020638~2
KS080932-M-3	Farmec-19/KS020363WM~1KS06O3A~25
KS081057-K-1	KS010514-9TM-10/KS020363WM~1//KS06O3A~58
KS080942-K-3	X060514-11/KS06O3A~4//HV9W96-1271R-1/3/KS011020-6
KS081098-K-3	RAVI-8/OVERLEY//KS990160-4~5
KS081098-M-7	RAVI-8/OVERLEY//KS990160-4~5

Table 4-2. Percent *A. tosicella* biotype 1-induced folding and mean \pm CI number of biotype 1 on 8 Kansas State University advanced breeding lines compared with the mite-resistant control, OK05312 and the susceptible Jagger control at 14 days post - biotype 1 infestation.

Wheat genotype	% leaf folding	χ^2 Fisher's exact test		Mean \pm CI number of biotype 1
		OK05312	Jagger	
OK05312	0	-	ns	6.4 \pm (3.0, 13.5) b
AYN3-38	50	*	ns	113.6 \pm (55.6, 231.7) a
AYN3-37	90	**	ns	182.1 \pm (89.3, 371.1) a
AYN2-9	50	*	ns	184.1 \pm (90.3, 375.2) a
AYN2-37	50	**	ns	220.37 \pm (99.4, 488.4) a
AYN2-28	50	*	ns	222.1 \pm (108.9, 452.6) a
AYN2-36	50	*	ns	236.4 \pm (116.0, 481.7) a
AYN2-33	80	**	ns	255.8 \pm (125.5, 521.1) a
Jagger	60	*	-	284.7 \pm (139.7, 580.0) a
AYN3-34	70	**	ns	286.7 \pm (140.7, 584.0) a

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tukey's mean separation test). Means followed by a different letter differed significantly based on LSD mean separation test ($\alpha = 0.05$).

ns: not significant at $p > 0.05$; * significant at $p < 0.05$; ** significant at $p < 0.01$.

OK05312 = *Cmc4* resistant control; Jagger = susceptible control.

Table 4-3. Percent *A. tosichella* biotype 2-induced folding and mean \pm CI number of biotype 2 on 8 Kansas State University advanced breeding lines compared with the mite-resistant control, OK05312 and the susceptible Jagger control at 14 days post - biotype 2 infestation.

Wheat genotype	% leaf folding	χ^2 Fisher's exact test		Mean \pm CI number of biotype 2
		OK05312	Jagger	
OK05312	0	-	ns	27.0 \pm (14.1, 51.4) c
AYN3-37	60	ns	ns	45.2 \pm (22.1, 92.6) bc
AYN3-34	30	ns	ns	55.1 \pm (28.0, 108.1) bc
AYN2-28	40	ns	ns	91.0 \pm (48.1, 172.1) abc
AYN2-36	0	ns	ns	109.7 \pm (58.0, 207.3) abc
AYN2-33	40	ns	ns	138.8 \pm (73.4, 262.2) ab
AYN2-37	50	ns	ns	157.1 \pm (83.1, 296.7) ab
AYN2-9	20	ns	ns	165.8 \pm (87.7, 313.1) ab
AYN3-38	50	ns	ns	186.0 \pm (95.1, 363.4) ab
Jagger	40	ns	-	357.2 \pm (189.3, 673.8) a

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tukey's mean separation test). Means followed by a different letter differed significantly based on LSD mean separation test ($\alpha = 0.05$).

ns: not significant at $p > 0.05$; * significant at $p < 0.05$; ** significant at $p < 0.01$.

OK05312 = *Cmc4* resistant control; Jagger = susceptible control.

Discussion

Having information about how advanced breeding lines respond after arthropod infestations could: 1.) Help breeders select the best parents to incorporate several genes into new cultivars and increase the performance of wheat breeding against pests (Carrera et al. 2012; Liu et al. 2014); and 2.) Determine new sources of resistance to wheat curl mite (Harvey et al. 1999). This study found no advanced breeding lines resistant to biotype 1. That may be because biotype 1 has been prevalent in the wheat growing area for a longer time than biotype 2, or because the advance breeding lines need more development before release.

The results of the biotype 2 experiment showed that biotype 2 populations are significantly reduced by advanced breeding lines AYN3-37 and AYN3-34 compared to plants of the susceptible Jagger and are no different than the resistant, which suggests that they have biotype 2 resistance (Table 4-3). Additionally, the level of biotype 2 population reduction by advance breeding lines AYN2-28 and AYN2-36 was not significantly different from the resistant or susceptible controls, which suggests that these lines had intermediate resistance to biotype 2. Even though the advance breeding lines AYN2-33, AYN2-37, AYN2-9, and AYN3-38 had fewer biotype 2 compared to the susceptible control, there were no statistical differences among them, suggesting that they were susceptible to biotype 2. Finally, more sources of resistance to both biotype 1 and 2 are essential to reduce the impact of the yield losses in the wheat plants from the feeding of *A. tosichella* and as a vector of WSMV, HPWMoV, and TriMV in the U. S. North-Central Great Plains wheat producing region.

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Chapter 5 - General Summary and Conclusion

The wheat curl mite, *Aceria tosichella* Keifer, is a global pest of bread wheat that reduces wheat yields by direct damage and by transmission of Wheat Streak Mosaic- (WSMV), High Plains Wheat Mosaic- (HPWMoV), and Triticum Mosaic Virus (TriMV). To date, mite-resistant wheat genotypes are the only effective method to reduce the damage of the *A. tosichella* - virus complex. These genotypes have proven to be economically beneficial and ecologically safe. Nevertheless, U. S. Great Plains annual wheat production losses average 2% annually due to the *Aceria tosichella*-viruse complex.

The results provided in the preceding chapters provide valuable information to improve the management of *A. tosichella* by assessing the breadth of mite resistance (lack of virulence) in wheat varieties containing the *Cmc2*, *Cmc3*, and *Cmc4* mite resistance genes to *A. tosichella* populations throughout the U. S. Great Plains. Among the 25 mite populations assessed, 36% have overcome the resistance in *Cmc2*, while 24% have become virulent to *Cmc3*, which appears to remain effective against populations in Missouri and South Dakota. The mega-population consisting of all 25 mite sub-populations was avirulent to 80% of plants containing *Cmc4* or *Cmc4 + Wsm2* (WSMV) resistance genes. Thus, the level of resistance in *Cmc4* remains effective in suppressing selection for *A. tosichella* virulence, as indicated by the cultivars OK05312 (Oklahoma) and MT06X424 (Montana) containing *Cmc4* that appear to be suppressing *A. tosichella*-virus yield losses. Our results will provide accurate information that suggest the continued development and release of additional wheat varieties containing *Cmc4* based-resistance in the Great Plains. In addition, the identification of new sources of resistance to *A. tosichella* biotype 2 should encourage breeders to include these in new mite resistant genotypes.

WSMV was much more prevalent among the mite populations sampled than either HPWMoV or TriMV, occurring in and transmitted by mites from 76% of the populations sampled, compared to the occurrence of HPWMoV and TriMV, which were present in mites from only 16% and 8% of the locations, respectively. These results are similar to those of several previous studies of the presence of these viruses in both *A. tosicHELLa* and in plant foliage that demonstrate a higher WSMV frequency. Finally, our mite-based virus results are also similar to those reported in plants by Burrows et al. (2009), where neither study detected more than two viruses present at any one location, and the absence of all three viruses co-occurring in mites from any location. Thus, the use of mite-transmission of viruses is as accurate an indicator of virus presence in plants and may be more accurate, by allowing knowledge of the virus transmission pattern of a specific biotype

The determination and prediction of *A. tosicHELLa* biotypes is very important since biotype 2 is a more efficient vector of WSMV than biotype 1 and because WSMV enhances biotype 2 reproduction. In addition, biotype 2 has demonstrated the capacity to develop virulence to *Cmc3*. Thus, both factors will contribute to the efficacy of mite plant resistance and as a result, a direct bearing on *A. tosicHELLa* virulence management. Our results detected a continuum of biotype 1 : biotype 2 mixtures, ranging from 100% biotype 2 at two sites in Texas and two sites in Missouri, to 90% biotype 1 at one site each in Kansas and Missouri. These data provide the first accurate update in information about the distribution of *A. tosicHELLa* biotypes in the U.S. Great Plains since 1997 and demonstrate biotype variation within- and between wheat fields, and within the head of the same plant. These results, plus those that demonstrate the effects of precipitation and land cover on biotype distribution, suggest the need for comprehensive,

coordinated studies of *A. tosichella* biotype variation on at least a U. S. national basis, if not a global basis.

Appendix A - Supplementary data for Chapter 1

Supplementary Table 1. State, county, and GPS coordinates for locations of *A. tosicHELLa* samples collected.

Location		GPS Coordinate (Latitude, Longitude)			Collection date (mm.dd.yyyy)
State	County	Field 1	Field 2	Field 3	
KS	Saline	38.8622, -97.5715	38.8053, -97.7429	38.9061, -97.6477	05.21.2014
	Geary	39.0447, -96.9122	39.0587, -96.8585	38.9138, -96.6132	05.21.2014
	Finney	38.0591, -100.3221	38.0590, -100.4634	38.0590, -100.4634	06.04.2014
	Dickinson	38.6963, -97.2344	39.0385, -97.2163	39.0312, -97.2351	06.04.2014
	Ellsworth	38.7306, -98.2999	38.5659, -98.4440	38.8128, -98.2433	06.04.2014
	Greeley	38.2915, -101.7530	38.3643, -101.7530	38.4368, -101.7338	06.04.2014
	Barton	38.4845, -98.5546	38.3277, -98.8479	38.3277, -98.8479	06.04.2014
	Ellis	39.0449, -99.3167	38.9144, -99.3251	38.7405, -99.3176	06.04.2014
	SD	Hughes	44.5199, -100.4512	44.5187, -99.7029	44.5174, -99.9466
Lake		44.0660, -96.9491	44.0586, -97.0895	43.8998, -97.0693	06.25.2015
Tripp		43.4298, -99.8500	43.4174, -99.8506	43.4153, -99.8435	06.28.2015
ND	Ward	48.4441, -101.1904	48.4256, -101.1470	48.2694, -101.7068	07.08.2014
	Bottineau	48.6900, -100.3413	48.7666, -101.0703	48.7630, -101.0703	07.08.2014
NE	Cheyenne	41.3516, -102.7186	41.3080, -102.9381	41.3079, -102.9348	07.10.2014
	Hayes	40.4251, -101.0986	40.6475, -101.0463	40.6450, -101.0623	05.10.2015
	Furnas	40.0446, -100.1307	40.0093, -99.8934	40.1416, -99.8948	06.10.2015
	Saunders	41.3573, -96.5603	41.3575, -96.6660	41.3583, -96.6594	07.10.2015
MO	Barton	37.6177, -94.2938	37.6180, -94.3021	37.3982, -94.2887	07.12.2015
	Cape Girardeau	37.5424, -89.6553	37.5320, -89.6751	37.5743, -89.7299	07.12.2015
	Pike	39.3282, -90.9945	39.3295, -91.1364	39.2038, -91.3620	07.12.2015
	Pettis	38.7018, -93.1058	38.7052, -93.4210	38.7038, -93.4126	07.12.2015
	Stoddard	36.9748, -89.7614	36.9552, -90.0749	36.9582, -90.0748	07.12.2015
	Cooper	38.7890, -92.6514	38.8029, -92.8940	38.8029, -92.8939	07.12.2015
TX	Randall	-	-	-	-
	Dallam	-	-	-	-

Supplementary Table 2. Percentage of *Wheat Streak Mosaic Virus (WSMV)*, *High Plain Wheat Mosaic Virus (HPWMoV)* and *Triticum Mosaic Virus (TriMV)* detected positively in *A. tosichella* when infested with Jagger wheat plants collected from 25 locations in six states. n= 5 plants per each virus, total 15 plant/experiment. Comparison within a location only, one experiment/location.

State	County	% of Jagger plants testing positive for virus			χ^2	P Value
		WSMV	HPWMoV	TriMV		
Kansas	Barton	100	0	0	15.0	**
	Greeley	100	0	0	15.0	**
	Ellsworth	80	0	0	10.9	*
	Dickinson	100	0	0	15.0	**
	Geary	0	0	0	-	-
	Finney	100	0	0	15.0	**
	Saline	0	0	0	-	-
	Ellis	100	0	0	15.0	**
Nebraska	Cheyenne	80	0	0	10.9	*
	Hayes	100	0	0	15.0	**
	Saunders	0	0	0	-	-
	Furnas	0	0	0	-	-
South Dakota	Hughes	80	0	0	10.9	*
	Lake	100	60	0	10.1	**
	Tripp	80	60	0	6.9	ns
North Dakota	Bottineau	60	80	0	6.9	ns
	Ward	0	80	0	10.9	*
Texas	Dallam	100	0	0	15.0	**
	Randall	100	0	0	15.0	**
Missouri	Cape Girardeau	100	0	0	15.0	**
	Pettis	0	0	0	-	-
	Stoddard	100	0	0	15.0	**
	Barton	100	0	100	15.0	**
	Pike	100	0	100	15.0	**
	Cooper	80	0	0	10.9	*

*significant at $P < 0.01$; ** significant at $P < 0.001$; ns = non-significant at $P > 0.05$; - no virus frequency.

Supplementary Table 3. Mean \pm CI ELISA ratios ^a for *Wheat Streak Mosaic Virus (WSMV)*, *High Plains Wheat Mosaic Virus (HPWMoV)* and *Triticum Mosaic Virus (TriMV)* contained in *A. tosichella* populations from 25 counties in Kansas, Missouri, Nebraska, North Dakota, South Dakota, and Texas after 21 d of feeding on plants of *A. tosichella* susceptible Jagger wheat.

State	County	Virus		
		WSMV	HPWMoV	TriMV
Kansas	Barton	50.4 \pm (43.9, 57.8) a	0.6 \pm (0.1, 2.1) b	1.4 \pm (0.6, 3.2) b
	Dickinson	47.9 \pm (45.4, 50.3) a	2.0 \pm (-0.3, 4.5) b	2.4 \pm (0.0, 4.8) b
	Ellis	44.1 \pm (38.1, 51.1) a	1.7 \pm (0.8, 3.5) b	1.0 \pm (0.4, 2.7) b
	Ellsworth	47.7 \pm (29.5, 77.1) a	0.9 \pm (0.3, 2.8) b	1.5 \pm (0.6, 3.6) b
	Finney	56.0 \pm (49.2, 63.8) a	1.4 \pm (0.6, 3.2) b	2.3 \pm (1.2, 4.4) b
	Geary	1.5 \pm (0.7, 2.3) a	1.0 \pm (0.7, 1.4) a	1.3 \pm (1.1, 1.5) a
	Greeley	44.2 \pm (43.8, 44.6) a	0.9 \pm (0.5, 1.3) c	1.8 \pm (1.4, 2.2) b
	Saline	1.0 \pm (0.7, 1.3) b	2.3 \pm (2.1, 2.6) a	1.0 \pm (0.7, 1.2) b
	Missouri	Barton	28.0 \pm (24.3, 31.9) a	0.4 \pm (0.3, 0.5) c
Cape Girardeau		29.1 \pm (28.7, 29.5) a	2.0 \pm (0.2, 3.8) b	1.0 \pm (0.6, 1.3) b
Cooper		24.8 \pm (20.4, 30.2) a	1.0 \pm (0.4, 2.7) b	0.7 \pm (0.2, 2.2) b
Pettis		0.9 \pm (0.3, 2.5) a	1.8 \pm (0.9, 3.8) a	1.0 \pm (0.3, 2.6) a
Pike		30.3 \pm (25.4, 36.1) a	0.2 \pm (0.0, 1.7) c	8.5 \pm (6.1, 11.9) b
Stoddard		29.3 \pm (24.4, 35.1) a	1.4 \pm (0.6, 3.2) b	3.9 \pm (2.4, 6.5, 1.5) b
Nebraska	Cheyenne	47.6 \pm (41.3, 54.8) a	1.3 \pm (0.5, 3.0) b	2.2 \pm (1.1, 4.3) b
	Furnas	1.0 \pm (0.8, 1.1) a	0.7 \pm (0.6, 0.8) c	3.8 \pm 3.4, 4.3) b
	Hayes	26.7 \pm (22.1, 32.2) a	1.5 \pm (0.6, 3.3) b	0.4 \pm (0.0, 1.8) b
	Saunders	1.0 \pm (0.3, 2.6) a	1.0 \pm (0.4, 2.7) a	1.8 \pm (0.9, 3.8) a
North Dakota	Bottineau	45.6 \pm (39.5, 52.7) a	41.6 \pm (35.8, 48.4) a	0.9 \pm (0.3, 2.5) b
	Ward	1.0 \pm (0.4, 2.7) b	43.6 \pm (37.7, 50.6) a	0.6 \pm (0.1, 2.1) b
South Dakota	Hughes	28.3 \pm (17.3, 46.1) a	0.9 \pm (0.3, 2.8) b	1.1 \pm (0.4, 3.1) b
	Lake	29.0 \pm (18.2, 46.1) a	16.7 \pm (10.2, 27.3) a	1.2 \pm (0.4, 3.2) b
	Tripp	23.5 \pm (14.9, 37.2) a	22.1 \pm (13.9, 35.0) a	1.7 \pm (0.7, 4.0) b
Texas	Dallam	29.1 \pm (28.5, 29.8) a	1.7 \pm (1.0, 2.4) b	1.4 \pm (0.7, 2.1) b
	Randall	27.0 \pm (26.7, 27.4) a	1.6 \pm (1.2, 2.0) b	1.1 \pm (0.7, 1.5) b

Means within a row followed by the same letter are not significantly different ($P > 0.05$, Tukey's LS mean test)

^aELISA ratio = OD₄₀₅ value of infected leaf/OD₄₀₅ value of healthy uninfected leaf.

Supplementary Table 4. n, F, treatment and total degrees of freedom, and P values for mean \pm CI number of *A. tosichella* from 25 locations on plants containing the *Cmc2*, *Cmc3*, *Cmc4*, or *Cmc4 + Wsm2* genes. n= 5 plants per each genotype, total 25 plant/ experiment.

State	County	n	Total # experiments	F	Treatment df, total df	P <
Kansas	Barton	25	1	5.08	4, 20	0.005
	Greeley	25	1	5.51	4, 20	0.0037
	Ellsworth	25	1	5.60	4, 20	0.0034
	Dickinson	25	1	1.88	4, 20	0.1543
	Geary	25	1	11.15	4, 20	0.0001
	Finney	25	1	28.36	4, 20	0.0001
	Saline	25	1	5.39	4, 20	0.0041
	Ellis	25	1	7.42	4, 20	0.0008
Missouri	Cape Girardeau	25	1	4.92	4, 20	0.0063
	Pettis	25	1	45.85	4, 20	0.0001
	Stoddard	25	1	14.55	4, 20	0.0001
	Barton	25	1	10.65	4, 20	0.0001
	Pike	25	1	27.75	4, 20	0.0001
Nebraska	Cooper	25	1	4.01	4, 20	0.0151
	Cheyenne	25	1	6.82	4, 20	0.0012
	Hayes	25	1	25.47	4, 20	0.0001
	Saunders	25	1	38.02	4, 20	0.0001
North Dakota	Furnas	25	1	9.30	4, 20	0.0002
	Bottineau	25	1	11.94	4, 20	0.0001
South Dakota	Ward	25	1	3.80	4, 20	0.0186
	Hughes	25	1	14.54	4, 20	0.0001
Texas	Lake	25	1	14.49	4, 20	0.0001
	Tripp	25	1	12.12	4, 20	0.0001
	Dallam	25	1	6.89	4, 20	0.0012
	Randall	25	1	12.61	4, 20	0.0001

Supplementary Table 5. n, F, treatment and total degrees of freedom, and P values for ELISA ratios of Jagger wheat plants infested with *A. tosicHELLa* from 25 locations. n= 5 plants per each virus, total 15 plant/ experiment.

State	County	n	Total # experiments	F	Treatment df, total df	P <
Kansas	Barton	15	1	72.53	2, 12	0.0001
	Greeley	15	1	18407.2	2, 12	0.0001
	Ellsworth	15	1	45.64	2, 12	0.0001
	Dickinson	15	1	545.85	2, 12	0.0001
	Geary	15	1	2.03	2, 12	0.2125
	Finney	15	1	100.67	2, 12	0.0001
	Saline	15	1	42.16	2, 12	0.0001
	Ellis	15	1	77.61	2, 12	0.0001
Nebraska	Cheyenne	15	1	88.45	2, 12	0.0001
	Hayes	15	1	45.70	2, 12	0.0001
	Saunders	15	1	0.87	2, 12	0.4434
	Furnas	15	1	254.60	2, 12	0.0001
South Dakota	Hughes	15	1	32.50	2, 12	0.0001
	Lake	15	1	20.30	2, 12	0.0001
	Tripp	15	1	19.23	2, 12	0.0002
North Dakota	Bottineau	15	1	34.49	2, 12	0.0001
	Ward	15	1	61.99	2, 12	0.0001
Texas	Dallam	15	1	6721.49	2, 12	0.0001
	Randall	15	1	2562.11	2, 12	0.0001
Missouri	Cape Girardeau	15	1	7989.23	2, 12	0.0001
	Pettis	15	1	1.05	2, 12	0.3804
	Stoddard	15	1	61.50	2, 12	0.0001
	Barton	15	1	28330.1	2, 12	0.0001
	Pike	15	1	39.69	2, 12	0.0001
	Cooper	15	1	46.12	2, 12	0.0001

Appendix B - Supplementary data for Chapter 2

Supplementary Table 1. State, county, and GPS coordinates for locations of *A. tosicHELLa* samples collected.

Location		GPS Coordinate (Latitude, Longitude)			Collection date (mm.dd.yyyy)
State	County	Field 1	Field 2	Field 3	
KS	Saline	38.8622, -97.5715	38.8053, -97.7429	38.9061, -97.6477	05.21.2014
	Geary	39.0447, -96.9122	39.0587, -96.8585	38.9138, -96.6132	05.21.2014
	Finney	38.0591, -100.3221	38.0590, -100.4634	38.0590, -100.4634	06.04.2014
	Dickinson	38.6963, -97.2344	39.0385, -97.2163	39.0312, -97.2351	06.04.2014
	Ellsworth	38.7306, -98.2999	38.5659, -98.4440	38.8128, -98.2433	06.04.2014
	Greeley	38.2915, -101.7530	38.3643, -101.7530	38.4368, -101.7338	06.04.2014
	Barton	38.4845, -98.5546	38.3277, -98.8479	38.3277, -98.8479	06.04.2014
	Ellis	39.0449, -99.3167	38.9144, -99.3251	38.7405, -99.3176	06.04.2014
SD	Hughes	44.5199, -100.4512	44.5187, -99.7029	44.5174, -99.9466	06.20.2014
	Lake	44.0660, -96.9491	44.0586, -97.0895	43.8998, -97.0693	06.25.2015
	Tripp	43.4298, -99.8500	43.4174, -99.8506	43.4153, -99.8435	06.28.2015
ND	Ward	48.4441, -101.1904	48.4256, -101.1470	48.2694, -101.7068	07.08.2014
	Bottineau	48.6900, -100.3413	48.7666, -101.0703	48.7630, -101.0703	07.08.2014
NE	Cheyenne	41.3516, -102.7186	41.3080, -102.9381	41.3079, -102.9348	07.10.2014
	Hayes	40.4251, -101.0986	40.6475, -101.0463	40.6450, -101.0623	05.10.2015
	Furnas	40.0446, -100.1307	40.0093, -99.8934	40.1416, -99.8948	06.10.2015
	Saunders	41.3573, -96.5603	41.3575, -96.6660	41.3583, -96.6594	07.10.2015
MO	Barton	37.6177, -94.2938	37.6180, -94.3021	37.3982, -94.2887	07.12.2015
	Cape Girardeau	37.5424, -89.6553	37.5320, -89.6751	37.5743, -89.7299	07.12.2015
	Pike	39.3282, -90.9945	39.3295, -91.1364	39.2038, -91.3620	07.12.2015
	Pettis	38.7018, -93.1058	38.7052, -93.4210	38.7038, -93.4126	07.12.2015
	Stoddard	36.9748, -89.7614	36.9552, -90.0749	36.9582, -90.0748	07.12.2015
	Cooper	38.7890, -92.6514	38.8029, -92.8940	38.8029, -92.8939	07.12.2015
TX	Randall	-	-	-	-
	Dallam	-	-	-	-

Supplementary Table 2. State, county, and GPS coordinates for locations of *A. tosichella* samples collected

Location		GPS Coordinate (Latitude, Longitude) 3 sites / field			Collection date (mm.dd.yyyy)
State	County	Field 1	Field 2	Field 3	
MO	Barton	37.7352, -94.4532	37.6178, -94.2940	37.3983, -94.2949	06.11.2016
		37.7352, -94.4532	37.6178, -94.2958	37.3983, -94.2864	06.11.2016
		37.7412, -94.4527	37.6178, -94.2948	37.3994, -94.2861	06.11.2016
	Cape Girardeau	37.5420, -89.6559	37.5254, -89.6701	37.3993, -89.2861	06.12.2016
		37.5414, -89.6579	37.5265, -89.6701	37.6010, -89.7289	06.12.2016
		37.5426, -89.6550	37.5272, -89.6701	37.6022, -89.7291	06.12.2016
KS	Ellis	38.8992, -99.5546	38.8991, -99.5286	38.9082, -99.5210	06.16.2016
		38.8989, -99.5490	38.8994, -99.5257	38.9069, -99.5210	06.16.2016
		38.8992, -99.5502	38.8995, -99.5216	38.9092, -99.5210	06.16.2016
NE	Hayes	40.6365, -101.0449	40.5346, -101.0274	40.4155, -101.0305	06.16.2016
		40.6340, -101.0449	40.5346, -101.0255	40.4156, -101.0246	06.16.2016
		40.6387, -101.0450	40.5310, -101.0250	40.4156, -101.0273	06.16.2016