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Population Dynamics of the Wheat Curl Mite (Acari: Eriophyidae) During the Heading Stages of Winter Wheat

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Abstract

The wheat curl mite (*Aceria tosichella* Keifer) is the only known vector of three viruses in wheat—*Wheat streak mosaic virus*, *Wheat mosaic virus*, and *Triticum mosaic virus*. The economic impact of this disease complex is linked to the presence of suitable hosts prior to winter wheat maturing in early summer and the movement of wheat curl mite from wheat to overwintering hosts prior to wheat harvest. Previous research has documented the prevalence and density of mite populations on maturing wheat heads; however, these studies were limited to a few late stages of wheat. A study was conducted to evaluate mite population densities across all stages of head development to determine when wheat curl mites are most abundant and the relative increase in abundance over time. In addition, a study was conducted to evaluate the impact of rainfall on mite populations during wheat heading. A final study was conducted to determine the potential for direct infestation of seedlings germinating from wheat curl mite-infested wheat heads. Results showed a rapid buildup in mite populations from low densities in early heading and peaking at the hard dough stage, with nearly all wheat heads having some mite presence. In addition, high mite populations resulted in direct infestation of germinated seedlings from the early through hard dough stages. Rainfall applications had no observable impact on mite population densities in wheat heads. These results demonstrate the increased potential for mites to infest hosts prior to winter wheat maturing and illustrate the increased risk for these hosts to serve as overwintering hosts.

Keywords: wheat curl mite, *Aceria tosichella*, winter wheat, rainfall, plant virus

Wheat is a staple food crop worldwide, and it is a core component of many dryland cropping systems in the western Great Plains of North America. The wheat–mite–virus complex is a consistent and significant threat to wheat production in this region. During the 2015 season, Kansas estimated yield losses of ~11 million bushels (2.7%) across the state from this disease complex (Appel et al. 2015). This complex consists of three viruses (*Wheat streak mosaic virus*, *Triticum mosaic virus*, and *Wheat mosaic virus*) that are transmitted to wheat by the wheat curl mite, *Aceria tosichella* Keifer.

Yield impacts from this disease complex are not equally distributed throughout the Great Plains region. Significant yield losses are often concentrated in localized areas where volunteer wheat has emerged prior to wheat harvest (preharvest volunteer; Staples and Allington 1956, Wegulo et al. 2008). This preharvest volunteer wheat most often results from severe hail storms occurring during the heading stages of winter wheat. Hail dislodges immature seeds from wheat heads; these seeds germinate in the presence of adequate moisture, and mites move via wind from maturing wheat heads to this newly germinated volunteer wheat. Once the volunteer wheat is infested, mite populations can build throughout the summer months. In the fall, mites disperse from the volunteer wheat to adjacent newly planted wheat fields, and they transmit viruses to the wheat, causing significant yield losses (Staples and Allington 1956, Wegulo et al. 2008).

The potential for mite infestation and virus impact on fall-planted winter wheat is strongly linked to the presence of viable hosts for mites that become infested prior to wheat harvest (Staples and Allington 1956, Wegulo et al. 2008). This temporal overlap in hosts is important for the epidemiology of the wheat–mite–virus complex owing to the limited off-plant survival of wheat curl mites. According to Wosula et al. (2015), the maximum time period for mite survival without a host is 7 d at 10 °C and 95% humidity, but at low humidity, survival is reduced to 2 d. However, increasing temperatures to 30°C reduced survival to 30 and 6 h for high and low humidity, respectively (Wosula et al. 2015). In western Nebraska, mean July temperatures over the past 30 yr are typically around 23 °C, with maximum temperatures around 30 °C (High Plains Regional Climate Center—University of Nebraska). This limited off-plant survival increases the importance of understanding the characteristics of mite build up on wheat heads and their ability to transition to a suitable overwintering host.

Previous studies have documented the abundance and presence of wheat curl mites on wheat heads at the soft and hard dough stages of head development. Mahmood et al. (1998) found that randomly selected wheat heads from fields in western Nebraska averaged 1,203 mites per head in 1995 and 487 mites per head in 1996 (Mahmood et al. 1998). Mite populations varied significantly between wheat fields, with means ranging from 23 to 1,872 mites per head (Mahmood et al. 1998). Byamukama et al. (2016) collected wheat heads from fields from three distinct regions across Nebraska and found greater mite populations in the Panhandle (380 mites per head) compared with west-central (200 mites per head) and southeast (50 mites per head) during the 2011 growing season. This pattern followed a precipitation gradient present across Nebraska from ca. 400mm mean annual rainfall in the west to ca. 800mm in the east (National Oceanic and Atmospheric Administration [NOAA] 2016). In 2012, greater mite numbers were observed across all regions of the state ranging from 800 to 1,200 mites per head; however, no significant differences were found across the three regions (Byamukama et al. 2016). Both of these studies documented wide fluctuations in the mean number of mites between years across a broad geographic region, indicating that certain environmental factors may be important for determining mite population densities on wheat heads. The relative increase in mean number of mites per wheat head across Nebraska between the 2011 and 2012 seasons coincided with widespread drought in 2012. Perhaps, the frequency or abundance of rainfall during heading stages could be an important factor for determining mite population densities on wheat heads. If this is the case, the gradient in rainfall patterns across Nebraska would indicate that greater mite populations may be present under drier climates in the west. However, observations on the correlation between rainfall and mite populations are confounded by an increasing number of wheat acres over this same geographic region. In addition, the variation in precipitation patterns for specific fields makes interpretations of rainfall impacts difficult, indicating the need for specific studies to evaluate the impact of rainfall on mite populations in headed wheat. No studies have evaluated the impact of rainfall on mite populations during the vegetative or reproductive stages of winter wheat.

Mite population densities have been found to be an important component for determining mite movement. A study by Thomas and

Hein (2003) found a strong relationship between increasing mite population densities on wheat plants and the number of mites moving off of wheat plants. Other studies have documented a flush of mites off of wheat following glyphosate application during vegetative stages of wheat development (Brey 1998). For reproductive stages of wheat, mite movement off of plants has been correlated with the senescence of flag leaves and wheat heads (Nault and Styer 1969).

Previous research has documented the prevalence and density of mite populations on maturing wheat heads; however, these studies have been limited to the soft and hard dough stage of wheat. No information is currently available on the seasonal dynamics of mite populations on wheat heads. The objective of this study was to evaluate mite population densities at different stages of wheat head development to determine the wheat stages when wheat curl mites are most abundant and the relative increase in mite populations across those development stages. In addition, wheat heads collected from these same fields were evaluated for the potential for mites to infest germinated wheat seedlings directly from infested grain under isolated conditions. A final study was undertaken to determine the impact of rainfall on mite populations during the heading stages of wheat.

Materials and Methods

Mite Dynamics in Wheat Heads

Wheat heads were collected from fields over three separate growing seasons at two locations per season, in conjunction with the Winter Wheat State Variety Trials conducted by the University of Nebraska-Lincoln. The 2011–2012 and 2013–2014 samples were collected from Cheyenne and Deuel County, NE. The 2012–2013 samples were collected from Cheyenne and Kimball County. Four wheat varieties (Pronghorn, Mace, Millennium, and Camelot) were sampled during the 2011–2012 and 2012–2013 growing seasons, whereas only two varieties (Pronghorn and Camelot) were sampled in 2013–2014. Wheat varieties were grown in a randomized complete block design with five replications. Each plot consisted of six 6-m rows, with a 0.3 m spacing between rows. Plots were sampled every 7–9 d beginning at the water ripe stage until harvest, with 5–8 collections occurring during

each season. For each sample, five wheat heads were randomly selected from the far-right row of each plot to avoid having an impact on whole plot yields. Wheat heads were cut 1–2 cm below the lowest spikelet, placed in plastic bags, and placed on ice. Heads were individually staged based on a seed selected from the middle of each wheat head. After staging, two of the five heads were placed on high definition tape that was secured to black cardstock (7-cm by 29-cm) with a double sided tape to determine WCM population per head (Harvey and Martin 1988, Byamukama et al. 2016). Awns of wheat heads were firmly pressed against the tape to ensure contact. Wheat heads were placed in plastic shoe boxes and covered with lids to prevent air movement around the heads for a period of 6 wk before counting. Mite counts on the tape were made by using a stereomicroscope at 30–40× magnification. Total heads collected varied between seasons with 400, 440, and 260 heads counted during 2012, 2013, and 2014.

For the remaining three wheat heads, the awns of each head were clipped back to the glumes, seeds were mechanically separated and spread into individual clear plastic clamshell containers (10 by 10 cm) containing 30 grams of sterilized greenhouse soil (2-cm soil depth). The soil surface was sprayed with 12ml of distilled water and sealed. Containers were held at 18–24 °C and five randomly selected plants were harvested from germinated containers at 21 d to determine mite presence. Mite presence was evaluated under a stereomicroscope at 30–40×. In total, 600, 660, and 390 heads were placed on soil for germination during the 2012, 2013, and 2014 season.

Rainfall Study

A simulated rainfall study was conducted over 2 yr (2013, 2014) in commercial wheat production fields planted to 'Settler CL' at the University of Nebraska's High Plains Agricultural Lab near Sidney, NE. The study consisted of four artificial rainfall applications (no rain, early application, late application, and both early and late application) in a randomized complete block design with six replications. Each plot consisted of four wheat rows with a 0.3 m row spacing and row lengths of 2.4 m.

Wheat plants were artificially infested with mites 3 wk prior to the first rainfall application during each season to increase mite numbers and the frequency of infested heads. In 2013, half of the replications

were infested with mites from a field with preharvest volunteer wheat. Volunteer wheat plants were cut at soil level and inspected at 30–40× magnification under stereomicroscope for mites. Plants were cut into 2–4-cm leaf sections, containing 30–40 mites per leaf piece. An individual leaf section was attached to each of 15 randomly selected wheat heads in the center two rows of each plot at wheat flowering. Metal paper clips were used to attach infested leaves, and tags were placed on the stems of each infested head. In 2014, mites were reared on 'Millennium' wheat in pots under greenhouse conditions for 4 wk prior to field infestation. Individual wheat plants contained >1,000 mites per plant at the time of field infestation. To infest field plots, individual wheat plants from pots were cut at the soil level and placed on the top of wheat plants in the field during the boot stage. The middle two rows of each plot were infested by laying the infested wheat end-to-end, covering ~1 m per row.

A rainfall simulator, designed by Meyer and Harmon (1979) was used to apply rain during the heading stages of winter wheat to evaluate the impact of rainfall on mite populations. A gas-powered Honda WB20XT water pump provided water pressure through a 15.8-mm garden hose at 41kpa and a height of 3 m from the soil surface, as suggested by Meyer and Harmon (1979). Aluminum catch pans on either side of the application area collected excess water and distributed it away from the study site. Teejet nozzles (80–150) passed between catch pans in ~0.5-s passes, with the duration of time spent in each catch pan determining the rainfall rate per hour. The machine was calibrated to apply 19mm of rain in 8min per rainfall treatment during the 2013 season and 25mm of rain in 11 min per rainfall treatment during the 2014 season. Wheat heads were collected every 10–12 d beginning at early milk stage and occurred prior to and following rainfall applications to measure the impact of rainfall on mite populations, with a total of 30 heads per rainfall treatment (5 per plot) for each of four collections per season. Heads were kept at 4 °C until they could be placed on high definition tape, as described previously.

Statistical Analysis

Winter wheat variety trial mite count data were analyzed by using PROC GLIMMIX (using SAS software version 9.3 (SAS Institute Inc., Cary, NC)) with repeated measures using the mean number of wheat

curl mites per plot for each stage of head development. No differences occurred between varieties; therefore, means were taken across varieties prior to the analysis. Fixed effects were wheat development stage and site nested within year. Years and sites were not analyzed separately because not all sites were represented during each year of the study. Random effects were replications. Least significant mean differences were used to determine differences within and between main effects. Proportion of infested wheat heads was also reported to determine the frequency of infested heads in wheat fields for each development stage and site \times year combination. The direct infestation of germinated seedlings was reported as a percentage of total plants evaluated. No statistical analysis was conducted on these data owing to the low frequency of infested plants.

Mite count data from the rainfall study were analyzed by using the same methods as described for the winter wheat variety trial, with repeated measures to test the fixed effects of rainfall application, collection date, and infestation method (2013 only). Random effects were collection date and replication. Mite counts from wheat heads were averaged for each treatment plot prior to analysis. Variances increased geometrically as a function of the mean. As a result, a negative binomial distribution was added to the model statement. Covariance models on inference (compound symmetry, autoregressive (1), antedependence (1), and unstructured) were tested to determine the model with the lowest Akaike information criterion corrected value, and degrees of freedom were adjusted using Kenward and Rogers methods to reduce test statistics biases. Environmental data were obtained from the High Plains Regional Climate Center (hprcc.unl.edu; University of Nebraska-Lincoln). Weather data originated from an established weather station located <2 km from the plot site.

Results

Mite Dynamics in Wheat Heads

The number of wheat curl mites per wheat head (Fig. 1) varied for each site year combination ($F = 15.15$; $df = 5, 94$; $P < 0.0001$), with the greatest mean occurring in Deuel County during 2012 (590) and 2014 (218) followed by Kimball County (94) in 2013. Mean number of mites

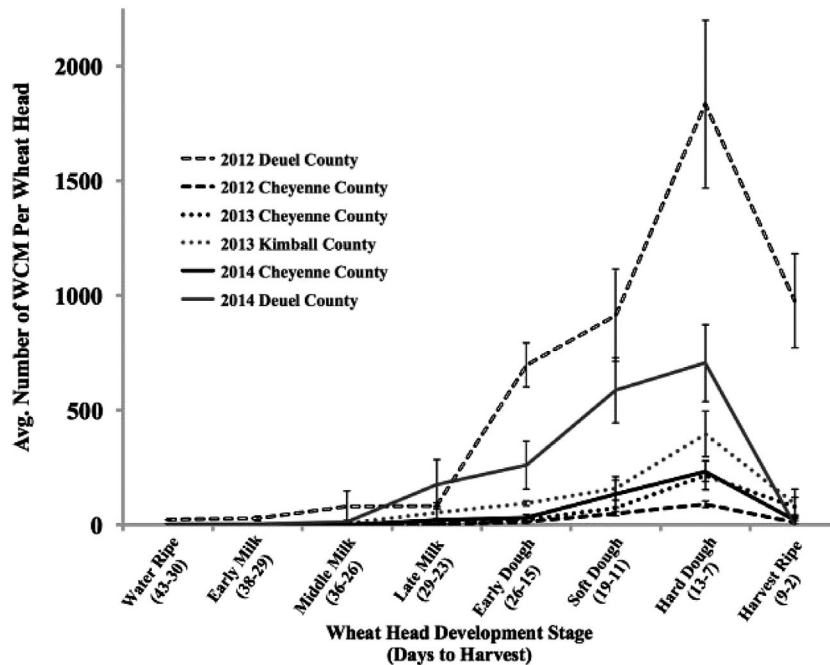


Fig. 1. Average number of wheat curl mites per head during different heading stages of wheat (water ripe, early milk, middle milk, late milk, early dough, soft dough, hard dough, and ripe) across site years (2012 Deuel, 2012 Cheyenne, 2013 Cheyenne, 2013 Kimball, 2014 Cheyenne, and 2014 Deuel).

per head was lowest in Cheyenne County at 20, 48, and 55 mites per head for 2012, 2013, and 2014, respectively. Mite numbers also varied by wheat development stage ($F = 21.74$; $df = 7, 408$; $P < 0.0001$), with the mean number of mites per head increasing from water ripe (1) through early milk (8), middle milk (12), late milk (39), early dough (267), soft dough (269), and hard dough (548) stages. Mite populations declined significantly ($t = 5.62$; $df = 408$; $P < 0.0001$) between the hard dough and harvest ripe (135) stages. A significant interaction occurred between site year and stage ($F = 6.68$; $df = 35, 408$; $P < 0.0001$) owing to the greater increase in mite populations at the hard dough stage for Deuel County during 2012 (1,819) and 2014 (730) compared with Cheyenne County during 2012 (89), 2013 (212), and 2014 (231) or Kimball County in 2013 (403). In contrast, mean mite populations for all collections were < 100 mites per head for all Counties and years, with the exception of Deuel County during 2012 at 1,010 mites per head.

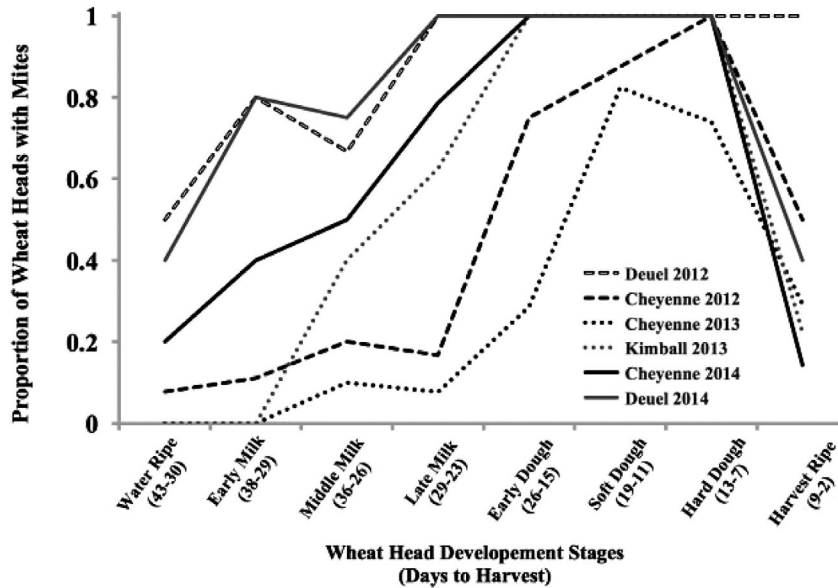


Fig. 2. Proportion of mite-infested wheat heads during different heading stages of wheat (water ripe, early milk, middle milk, late milk, early dough, soft dough, hard dough and ripe) across years (2012, 2013, and 2014) and locations (Cheyenne, Deuel, and Kimball Counties).

The proportion of wheat heads (Fig. 2) infested with mites reached 100% for every site year, with the exception of Cheyenne County during 2014 (84% infested). In addition, the greatest mite populations per wheat head occurred after the frequency of infested heads reached 100% during the middle milk stage. In contrast, lower mite populations per wheat head occurred when infestations of 100% did not occur until late milk to hard dough wheat stages. Sites with high mite populations also corresponded with a high proportion of infested wheat heads at water ripe stage, which was in excess of 40% for both Deuel County in 2012 and 2014.

Mite Infestation of Seedlings

Of the 4,037 plants evaluated, 61 plants were found to be infested with wheat curl mites, demonstrating that mites were able to directly infest germinated wheat seedlings from infested grains under controlled conditions. Seedling infestation from infested grain varied by site year and wheat development stage. Mites were found on

seedlings in four of the six site years, with the greatest percentage of mites occurring in Deuel County during 2012 at 5% (47/921) and 2014 at 2% (7/398). In Cheyenne County, only three and seven plants were found to be infested during 2012 and 2013, respectively. Of the seven stages of head development, mites were first observed during the early dough stage at 1% (8/1163), with increasing levels of infestation for soft at 2% (20/889) and hard at 7% (31/467) dough stages. Only two of 727 plants were found to be infested with mites during the harvest ripe stage.

Rainfall Study

Mite infestation method, natural rainfall, as well as application timing and amount of rain applied varied between the 2 yr of the study; therefore, each year was analyzed separately. In 2013, limited natural rainfall occurred (Fig. 3a) during wheat heading, with the exception of 45mm of rain on 18 June. More frequent rainfall occurred during the 2014 season (Fig. 3b) following the early rainfall application date, with nine of the 10 d after the application having measurable precipitation. However, the natural rainfall events during this 10-d period were low (2–15 mm). For the late season application in 2014, only 2 of the 9 d following the second rainfall application had precipitation with rainfall of <5 mm on either day.

An analysis of variance on mite population data during the 2013 season showed no significant infestation method interaction with rainfall treatments ($F = 0.10$; $df = 3, 17.7$; $P = 0.9564$); therefore, infestation methods were combined for the analysis. In 2013, mite populations on wheat heads (Fig. 4a) varied by collection date ($F = 18.86$; $df = 3, 20$; $P < 0.0001$), with increasing mite populations for collection one (1.5), two (16.2), and three (65). Mite populations declined in the final collection (13.6) period. Rainfall applications showed differences in mite populations ($F = 6.50$; $df = 3, 18$; $P = 0.0036$); however, these differences were not consistent with simulated rainfall treatments. The greatest number of mites across all collection dates occurred with early (51) and late (39) rainfall applications followed by no rainfall (29) and the combination rainfall application (22). The interaction between rainfall application and collection date was not significant ($F = 1.73$; $df = 9, 25.3$; $P = 0.1333$).

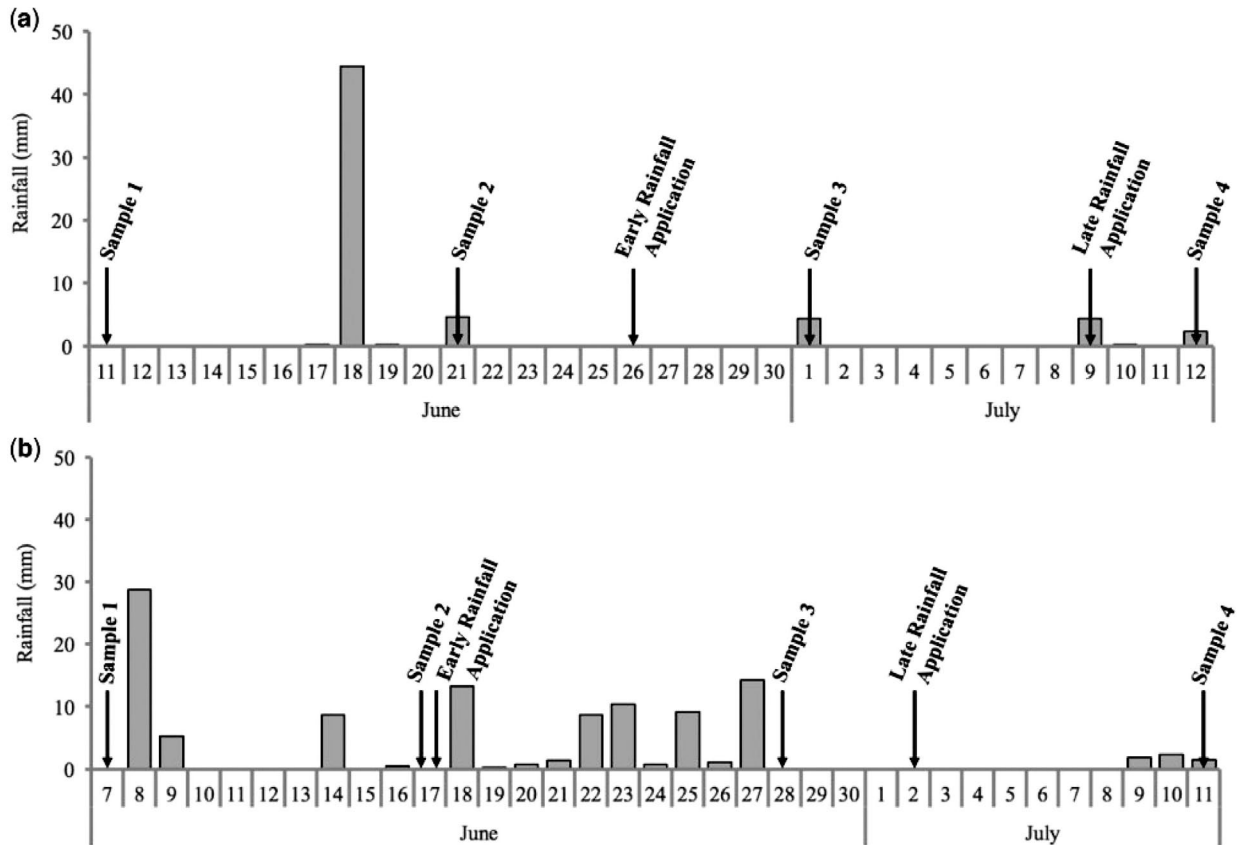


Fig. 3. Natural rainfall totals per day, wheat head sample (Sample 1, 2, 3, and 4) dates, and dates of rainfall applications (early and late) for 2013 (a) and 2014 (b) growing seasons. Wheat was infested on June 6th in 2013 and May 24th in 2014.

Artificial infestation of wheat heads during the 2014 season resulted in large mite populations on wheat heads, with some in excess of 16,000 mites per head (Fig. 4b). Mite populations on wheat heads varied by collection period ($F = 46.04$; $df = 3, 20$; $P < 0.0001$), with increasing mite populations from collection one (463), two (1,063), and three (6,054). Mite populations declined significantly ($t = 2.53$; $df = 20$; $P = 0.0200$) between collection dates three and four (4,484). No differences were observed between rainfall applications ($F = 0.72$; $df = 3, 18.1$; $P = 0.5502$) or for the interaction between rainfall application and collection date ($F = 0.73$; $df = 9, 25.4$; $P = 0.6819$).

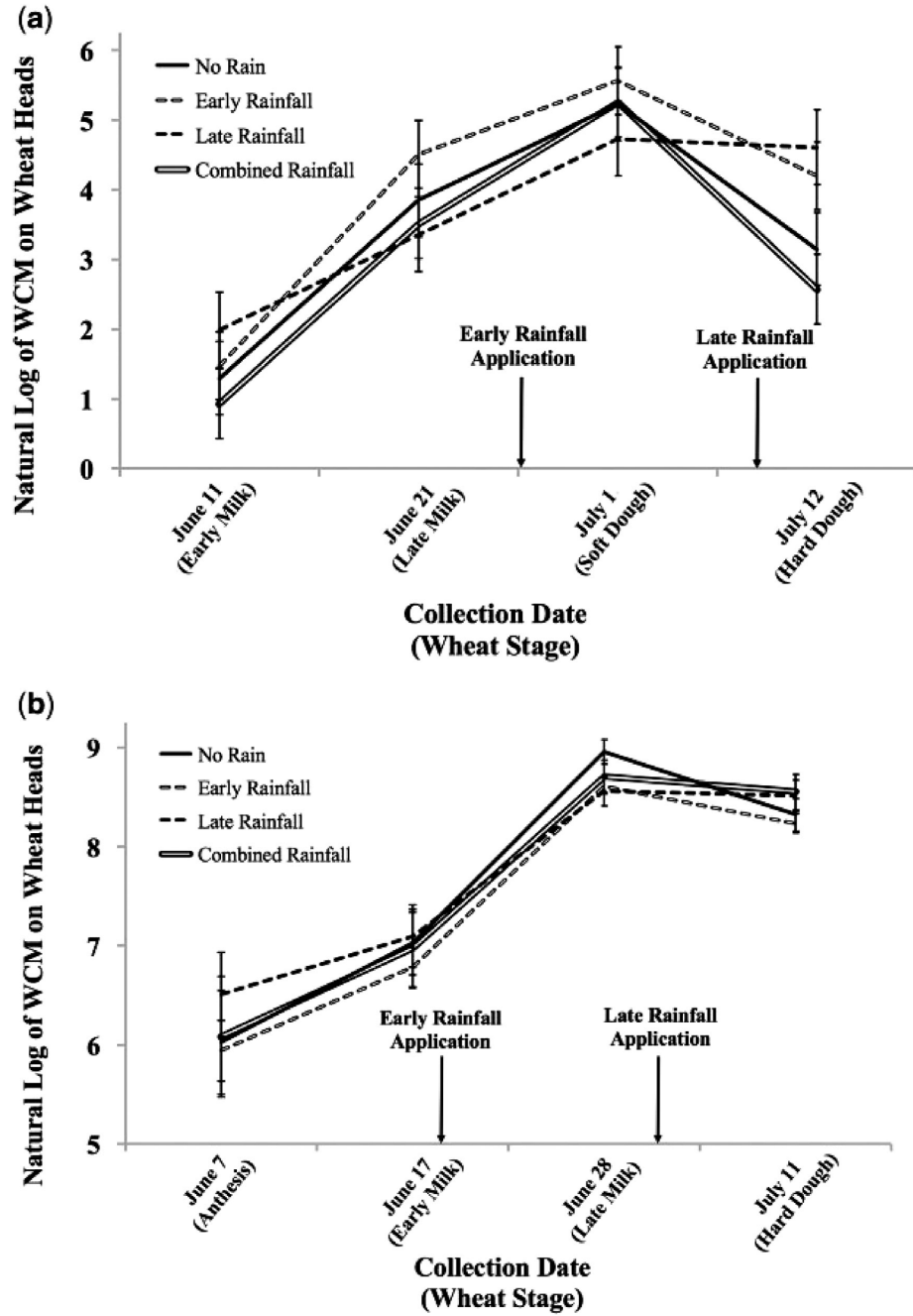


Fig. 4. Natural log of wheat curl mite populations on wheat heads across rainfall applications (no rain, early, late, and combined) and collection periods for simulated rainfall study during the 2013 (a) and 2014 (b) seasons.

Discussion

Mite populations on wheat heads (Fig. 1) collected from winter wheat variety trials in the western Panhandle of Nebraska varied considerably between site years. This variation and the mean number of mites per wheat head were consistent with results reported by Mahmood et al. (1998) and Byamukama et al. (2016). Regardless of the variation between site years, this study demonstrated a consistent and significant increase in mite populations as wheat heads advanced through development stages.

Early head collections from the water ripe through the late milk stages showed relatively low levels of mite populations. However, the proportion of infested heads at the water ripe stage (Fig. 2) varied considerably between locations at 0 and 50%, indicating a greater frequency of infested wheat heads in some fields soon after head emergence. During 2012 and 2014, we received and validated reports of significant yield losses from the wheat–mite–virus complex in Deuel County wheat fields within a 15-km radius of these field sites, indicating the potential for mite infestation during the previous fall. Mite populations at the Deuel County sites were the highest recorded for the study; however, even these populations did not coincide with a significant virus impact. Yield data from the winter wheat variety trials during 2012 show that virus resistant 'Mace' (1,550 kg/ha) had lower grain yields than commercially susceptible 'Camelot' (2,020 kg/ha), indicating a lack of significant pressure from wheat–mite–virus complex (Regassa et al. 2012). Mace was not present in 2014; however, yields for Camelot were at 3,030 kg/ha, indicating virus pressure was minimal (Regassa et al. 2014). In addition, no virus symptoms were observed in these variety trials. The lack of virus impact indicates that few mites were present through the fall and early spring, as virus impact is related to timing of infection (Hunger et al. 1992).

The results from this study demonstrate that not all wheat heads are infested with mites during the early stages of head development. This could be owing to the inability to detect low mite populations in wheat heads through the sticky tape method. The rapid and continued increase in the proportion of infested heads further supports the notion that low populations of mites were present within wheat heads. With the exception of Cheyenne County in 2013, all other site years

reached 100% infestation level, indicating that all wheat fields are likely to become infested with mites in the weeks prior to wheat harvest. Under the most conservative estimates, mean mite populations of 50 mites per head would result in mite populations of 269 million per hectare, assuming 164 heads per meter of wheat row. Nault and Styer (1969) suggested increasing mite movement from wheat fields with peak activity near harvest resulted from declining host suitability, but from this study, it is clear that increased mite movement during this time would have a direct connection to rapidly increasing mite populations as proposed by Thomas and Hein (2003).

Mite infestation of seedlings was also associated with high populations on wheat heads, with the highest mite populations in Deuel County accounting for 54 of the 61 plants with direct seedling infestation. This had not been previously documented. Direct mite infestation of wheat seedlings first occurred during the early dough stage, with an increasing number of infested plants through the soft and hard dough stages and a rapid decline at the harvest ripe stage. Controlled conditions likely increased mite survival owing to adequate moisture for seedling germination. Such conditions may be possible under field conditions, as hail damage typically destroys wheat stands, increasing the potential for dense residue shielding. Lower humidity levels are likely to decrease direct infestation of newly germinated wheat, owing to reduced mite survival (Wosula et al. 2015). This is apparent from the diminishing ability of mites to survive on harvest ripe wheat, as reflected in the decline in mite populations found in wheat heads and in reduced direct infestation on germinating seedlings. Greater seedling infestation by mites during soft and hard dough wheat stages indicates that mites continue to feed on seeds until germination occurs. This supports the idea that as wheat approaches harvest, seeds dry down rapidly and become unsuitable for mite feeding.

Rainfall applications had no consistent impact on mite populations during either year of the study. A few significant differences occurred between rainfall applications; however, these differences were not additive or consistent across treatment or years. In 2013, the highest mite populations occurred with the early and late rainfall applications, but the no rainfall and combined rainfall treatments had lower populations. The reduction in combined applications compared with the individual application suggests that some other factor besides rainfall

impacted mite populations. In addition, no significant interactions between rainfall application and collection days occurred, indicating a lack of impact on mite populations from rainfall treatments. In 2014, rainfall rates were increased to 25 mm; however, there were no differences between rainfall treatments or their interaction with collection dates, reinforcing the lack of impact from rainfall on mite populations.

The rainfall simulator used in this study was designed to produce raindrops of similar size and energy to those coming from natural rain events. This reproducibility of natural rainfall events demonstrates that rain during wheat heading likely has minimal impact on mite populations. A lack of impact on mite populations could be owing to the physical structure of wheat heads. The structure of the wheat head provides numerous protected niches for the mites and precludes raindrops from directly dislodging mites, and prevents water from collecting within the glumes of the wheat head where mites are typically found. Similar studies are needed to address the impact of rainfall on mite populations during the earlier, vegetative stages of wheat development when the mites are not protected within the heads.

The results from this study demonstrate the rapid seasonal buildup of mite populations during wheat heading, with peak populations occurring during the hard dough stage of winter wheat. Large populations of mites on winter wheat prior to harvest reinforced the need for producers to manage preharvest volunteer wheat or other potential overwintering hosts that can be infested at this time. In addition, we demonstrated that mites can directly infest germinated wheat from infested grains; however, this occurred at low levels and was limited to late stages of wheat development (early dough through hard dough). Our results show a lack of impact from rain applied during the heading stages of wheat, likely as a result of mites being protected from the direct impact of rain drops. These results increase our understanding of the factors that affect mite population dynamics in heading wheat and the potential for mites to infest suitable hosts prior to winter wheat maturing.

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