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REMOTE SENSING TO DETECT THE MOVEMENT OF WHEAT CURL MITES  
THROUGH THE SPATIAL SPREAD OF VIRUS SYMPTOMS, AND  
IDENTIFICATION OF THRIPS AS PREDATORS OF WHEAT CURL MITES

by

Abby R. Stilwell

A DISSERTATION

Presented to the Faculty of

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For the Degree of Doctor of Philosophy

Major: Entomology

Under the Supervision of Professors Gary L. Hein and Stephen D. Danielson

Lincoln, Nebraska

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REMOTE SENSING TO DETECT THE MOVEMENT OF WHEAT CURL MITES  
THROUGH THE SPATIAL SPREAD OF VIRUS SYMPTOMS, AND  
IDENTIFICATION OF THRIPS AS PREDATORS OF WHEAT CURL MITES

Abby Rose Stilwell, Ph.D.

University of Nebraska, 2009

Advisors: Gary L. Hein and Stephen D. Danielson

The wheat curl mite (WCM), *Aceria tosichella* Keifer, transmits three viruses to winter wheat: wheat streak mosaic virus, High Plains virus, and Triticum mosaic virus. This virus complex causes yellowing of the foliage and stunting of plants. WCMs disperse by wind, and an increased understanding of mite movement and subsequent virus spread is necessary in determining the risk of serious virus infections in winter wheat. These risk parameters will help growers make better decisions regarding WCM management. The objectives of this study were to evaluate the capabilities of remote sensing to identify virus infected plants and to establish the potential of using remote sensing to track virus spread and consequently, mite movement.

Although the WCM is small and very hard to track, the viruses it vectors produce symptoms that can be detected with remote sensing. Field plots of simulated volunteer wheat were established between 2006 and 2009, infested with WCMs, and spread mites and virus into adjacent winter wheat. The virus gradients created by WCM movement allowed for the measurement of mite movement potential with both proximal and aerial remote sensing instruments. The ability to detect WCM-vectored viruses with remote sensing was investigated by comparing vegetation indices calculated from proximal remote sensing data to ground truth data obtained in the field. Of the ten vegetation

indices tested, the red edge position (REP) index had the best relationship with ground truth data.

The spatial spread of virus from WCM source plots was modeled with cokriging. Virus symptoms predicted by cokriging occurred in an oval pattern displaced to the southeast. Data from the spatial spread in small plots of this study were used to estimate the potential sphere of influence for volunteer wheat fields.

The impact of thrips on WCM populations was investigated by a series of greenhouse, field, and observational studies. WCM populations in winter wheat increased more slowly when thrips populations were higher, both in the field and in the greenhouse. Two species of thrips, *Thrips tabaci* Lindeman and *Frankliniella occidentalis* (Pergande) were observed to feed directly on WCMs. The collective results from this study identify thrips as a regulating factor for WCM populations.

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## TABLE OF CONTENTS

ACKNOWLEDGEMENTS .....	i
LIST OF FIGURES .....	viii
LITERATURE REVIEW .....	1
Wheat Curl Mite Biology and Ecology .....	2
Transmission of Diseases .....	4
Alternate Hosts of the Wheat Curl Mite, Wheat Streak Mosaic Virus, High Plains Virus and Triticum Mosaic Virus .....	11
Management of the wheat curl mite .....	12
Wheat Curl Mite Movement and Risk Prediction .....	19
Remote Sensing .....	22
Literature Cited .....	32
CHAPTER 1. <u>Hyperspectral Remote Sensing to Detect Symptoms Associated with</u> <u>Wheat Curl Mite-Vectored Viruses</u> .....	42
Introduction .....	43
Materials and Methods .....	46
Results .....	51
Discussion .....	54
Literature Cited .....	60
Tables .....	65

Figures .....	72
CHAPTER 2: <u>Spatial Pattern of Wheat Curl Mite Movement and Subsequent Virus Spread in Winter Wheat</u> .....	77
Introduction .....	78
Materials and Methods .....	82
Results and Discussion.....	89
Conclusions .....	101
Literature Cited .....	103
Tables .....	107
Figures .....	115
CHAPTER 3: <u>Thrips as Predators of Wheat Curl Mites in Winter Wheat Cropping Systems</u> .....	120
Introduction .....	121
Materials and Methods .....	125
Results and Discussion.....	130
Conclusions .....	140
Literature Cited .....	142
Tables .....	146
Figures .....	149
Appendix A: Volunteer wheat plot .....	162



Appendix B: Mean percent of wheat tillers in each sampling ring infested with wheat curl mites for each year and plot. ....	166
Appendix C: 2007-08 north plot. Camera is pointed southeast. ....	171
Appendix D: Number of hours wind originated from each direction between 27 September and 30 November. ....	174
Appendix E: Mean $\pm$ standard error (SE) number of thrips adults, larvae, and total thrips per 25 wheat tillers in 2007. ....	175
Appendix F: Mean $\pm$ standard error (SE) number of thrips adults, larvae, and total thrips per 25 wheat tillers in 2008. ....	176
Appendix G: Mean $\pm$ standard error (SE) number of thrips adults, larvae, and total thrips per 25 sweep samples in 2008. ....	178

## LIST OF TABLES

1.1. Vegetation indices used in this study.....	66
1.2. Correlation between biophysical variables associated with virus symptoms.....	67
1.3. Coefficients of determination for vegetation indices regressed on biophysical variables for the north plot in the 2007-08 growing season.....	68
1.4. Coefficients of determination for vegetation indices regressed on biophysical variables for the south plot in the 2007-08 growing season.....	69
1.5. Coefficients of determination for vegetation indices regressed on biophysical variables for the 2008-09 growing season.....	70
1.6. Regression models for the red edge position vegetation index regressed on biophysical variables.....	71
2.1. Fall populations of wheat curl mites in source volunteer plots .....	108
2.2. Average hourly wind direction at variable wind speeds.....	109
2.3. Degree of association between biophysical variables and the red edge position vegetation index.....	110
2.4. Cross-validation between biophysical variables and the red edge position vegetation index.....	112
2.5. Approximate distance of virus spread from the center of the source plot in each cardinal and ordinal direction.....	113
2.6. Area outside the wheat curl mite source plot predicted by cokriging to be associated with each percent virus infection rate.....	114
3.1. Species of adult thrips collected in 2007 and 2008 by location.....	147

3.2. Mean number of thrips collected from greenhouse plants infested thrips.....	148
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## LIST OF FIGURES

1.1. Reflectance spectra of samples containing various wheat streak mosaic virus infection levels.....	73
1.2. Relationship between relative chlorophyll content and the red edge position.....	74
1.3. Relationship between leaf area index and the red edge position.....	75
1.4. Relationship between percent virus infection and the red edge position.....	76
2.1. Reflectance spectra of samples containing various wheat streak mosaic virus infection levels.....	116
2.2. Percent virus infection surrounding the 2007-08 north source plot predicted by cokriging.....	117
2.3. Percent virus infection surrounding the 2007-08 south source plot predicted by cokriging.....	118
2.4. Percent virus infection surrounding the 2008-09 source plot predicted by cokriging.....	119
3.1. Mean number of thrips per 25 tillers by date in each field and county in 2007.....	150
3.2. Mean number of thrips per 25 tillers by date in each field and county in 2008.....	151
3.3. Mean number of thrips per 25 sweeps by date in each field and county in 2008.....	152
3.4. Mean number of wheat curl mites per container in greenhouse plants infested with thrips and not infested with thrips in experiment 1.....	153

3.5. Mean number of wheat curl mites per container in greenhouse plants infested with thrips and not infested with thrips in experiment 2.....	154
3.6. Mean number of wheat curl mites per container in greenhouse plants infested with thrips and not infested with thrips in experiment 3.....	155
3.7. Mean number of wheat curl mites per container in greenhouse plants infested with thrips and not infested with thrips in experiment 4.....	156
3.8. Mean number of wheat curl mites per tiller in plots treated with insecticide to control thrips and untreated plots in 2006.....	157
3.9. Mean number of thrips per tiller in plots treated with insecticide to control thrips and untreated plots in 2006.....	158
3.10. Mean number of wheat curl mites per tiller in plots treated with insecticide to control thrips and untreated plots in 2008.....	159
3.11. Mean number of thrips per tiller in plots treated with insecticide to control thrips and untreated plots in 2008.....	160
3.12. Mean number of thrips per 10 plants in plots treated with insecticide to control thrips and untreated plots in 2008.....	161

## LITERATURE REVIEW

## Wheat Curl Mite Biology and Ecology

The wheat curl mite, *Aceria tosichella* Keifer, is a microscopic eriophyid mite widely distributed in North America (Oldfield 1970). Members of the Eriophyoidea have a worm-like body with transverse rings and possess two pairs of legs that distinguish them from other members of the Acari (Meyer 1981, Lindquist 1996). The wheat curl mite (WCM) measures approximately 250 microns by 75 microns (Keifer 1938), and a single mite is barely visible to the naked eye. The WCM undergoes growth stages typical of other eriophyids, with two nymphal stages occurring between the egg and the adult stage (Staples and Allington 1956) and a quiescent or resting period prior to each molt (Manson and Oldfield 1996). Quiescent forms are incapable of movement and appear translucent anteriorly and sometimes posteriorly (Staples and Allington 1956).

Females are inseminated after picking up a spermatophore deposited on the host plant surface by males (Oldfield 1970, Sternlicht and Goldberg 1971). Fertilized females produce haploid males and diploid females. Females reared in the absence of males can produce haploid males by arrhenotoky parthenogenesis (Helle and Wysoki 1983). Eggs are laid along leaf veins (Peairs 2006). Each female lays at least 12 eggs and the complete cycle, from egg to egg, typically takes 8 to 10 days (Staples and Allington 1956). WCM development is temperature dependent. All stages can survive for several days at 0°F and at least three months at near freezing temperatures (Staples and Allington 1956). Mites don't appear to be negatively affected by extended cold winters in western Nebraska, and readily survive further north into Canada. Populations of mites increase most rapidly at temperatures between 24°C and 27°C. Humidity is important to WCM

survival and is directly related to the micro-environment supplied by the leaves (Slykhuis 1955). Humidity and temperature appear to be linked, in that mites are better able to survive high temperatures if humidity is also high (G.L. Hein, unpublished). Theoretically, one female is capable of producing three million offspring in as little as 60 days (Somsen and Sill 1970).

Eggs, immature stages, and adult WCMs are found on winter wheat and other nearby annual grasses (Peairs 2006). Upon arrival on a wheat plant, the WCM migrates to the base of the leaf sheath and into the whorl of a developing leaf to begin feeding (Orlob 1966a, Somsen and Sill 1970). Typically, WCMs develop in protected areas such as curled leaves or the leaf whorl, axil or sheath and eventually on green tissues in the head (Peairs 2006). As wheat heads emerge, mites move to the florets and developing kernels. The eggs of WCMs are most abundant in the sheltered areas near the base of the leaf, the ligule, and on wheat kernels when heading begins.

WCMs are able to penetrate the cuticle of leaves with their stylets and feed on cells just below the leaf surface (Sabelis and Bruin 1996). WCMs feed between leaf veins in the grooved sections that consist of epidermal tissue known as bulliform cells (Styer and Nault 1996). Feeding by the WCM prevents bulliform cells from enlarging and consequently, the actively growing leaf is prevented from uncurling. Mites may cause rolling of the entire leaf and the subsequent leaf to become trapped; causing curled, looped, and trapped leaves (Slykhuis 1955, Somsen and Sill 1970, Styer and Nault 1996). Rolling of the leaf causes wheat leaves to be more rigid and stand higher in the field, making them visible from a distance (Somsen and Sill 1970).



Damage to plants results from the withdrawal of nutrients, reduction of gas exchange and photosynthesis, death of epidermal cells, and distortion of plant or leaf growth (Sabelis and Bruin 1996). Heavy infestations of the WCM may reduce yield. At levels of 450 WCMs per wheat spike, yield may be reduced by 1% and natural infestations may cause losses from less than 1% to more than 15% (Harvey et al. 2000). Although the wheat curl mite has a direct effect on wheat yield, much greater damage and yield loss is associated with the virus complex it transmits.

### **Transmission of Diseases**

The WCM is responsible for transmitting three viruses to wheat, *Triticum aestivum* L.: wheat streak mosaic virus (WSMV) (Slykhuis 1953a, Slykhuis 1953b, Slykhuis 1955), High Plains virus (HPV) (Seifers et al. 1997), and Triticum mosaic virus (TriMV) (Seifers et al. 2008). All three viruses are responsible for causing damage to wheat. Plants infected with WSMV are hard to differentiate from plants infected with HPV or TriMV in the field, and infections of wheat by multiple viruses can occur in the same field (Mahmood et al. 1998, Seifers et al. 2009). The virus complex transmitted by the WCM is still under investigation and much more information is known about WSMV than HPV or TriMV.

### **Wheat Streak Mosaic Virus**

Wheat streak mosaic is potentially the most devastating disease of winter wheat in the central Great Plains and losses can range from negligible to complete crop failures (Hershman 2000). Outbreaks of this disease have caused extensive monetary loss (Sill and Agusiobo 1955, University of Illinois Extension 1989, Hershman 2000). According

to the Kansas Cooperative Plant Disease Survey Report, that is produced annually, losses due to wheat streak mosaic were 2.0% in 2005 and 18.1% in 2006 (Appel et al. 2005, 2006). The wheat loss reported in 2006 was the second worst loss since estimates began in 1976 (Appel et al. 2006) and the monetary loss calculated with data obtained from the National Agricultural Statistics Service (NASS) was approximately \$240.3 million (NASS 2006). Losses in 2007 and 2008 were less than 1.0% (Appel et al. 2007, Apple et al. 2008); however, averaged over twenty years, approximately 1.4% of wheat is lost annually in Kansas due to WSMV (Appel et al. 2008). Although no surveys have been conducted, average loss in Nebraska is similar to that of Kansas, mostly occurring in the western half of the state (G.L. Hein, personal communication). This estimate is likely a conservative estimate of loss because the majority of wheat grown in Nebraska is located in high-risk areas that are subject to frequent pre-harvest hail storms that increase the likelihood of subsequent virus infections.

WSMV was first reported in 1922 as yellow mosaic (Staples and Allington 1956) and has caused losses in locations throughout the world including central and western North America, Eastern Europe, parts of Russia, North Africa (University of Illinois Extension 1989) and Australia (Coutts et al. 2008b). Although other insect vectors were tested to determine if they transmitted WSMV, the WCM is the only vector able to transmit the virus to wheat (Connin and Staples 1957). All stages of the WCM except the eggs are able to carry and transmit WSMV when reared on diseased wheat (Slykhuis 1955, Siriwetwivat 2006); however, the WCM can only acquire the virus in the nymphal stage (Slykhuis 1955). WCMs that acquire the virus as nymphs are able to transmit it as adults (del Rosario and Sill 1965). Studies by Siriwetwivat (2006) determined the

efficiency of WSMV transmission by transferring eggs, nymphs, quiescent, and adult mites from infected plants to healthy plants. No plants were infected when eggs were transferred to healthy plants. When adults were transferred to healthy plants, 52% became infected with WSMV. When nymphs were transferred to healthy plants, 83% of plants were infected. When quiescent mites were transferred to healthy plants, 94% of plants were infected. In the absence of infective feeding material, the WCM remains viruliferous for up to 18 days; however, transmission of the virus is greatly reduced after 11 days (del Rosario and Sill 1965).

Large particles of the flexuous, rod-shaped virus accumulate in the midgut of the WCM and do not degrade for at least five days (Oldfield and Proeseler 1996).

Acquisition of WSMV by mites can occur after as little as 15 to 30 minutes of feeding (Orlob 1966a). Particles of WSMV have frequently been found in the haemocoel and salivary glands (Oldfield and Proeseler 1996).

It has been discovered recently that WSMV is also seed-borne (Jones et al. 2005). Seed transmission was found to be 0.2 to 0.5% across a wheat breeding collection in Australia, with up to 1.5% in individual genotypes. The consequences of seed transmission are that an incoming nonviruliferous mite can alight on seed-infected plants scattered within the wheat field, feed, acquire virus, and propagate. This method of transmission can cause circular patches of virus within fields (Coutts et al. 2008a), and may increase the risk of introduction of WSMV to new locations with the movement of germplasm (Jones et al. 2005, Dwyer et al. 2007) or increase the spread of the virus (Coutts et al. 2008b). Seed transmission may also be important to the spread of virus

isolates. The overall seed transmission rate in Australia is 0.5% (Jones et al. 2005); however, this low level of seed transmission would have little impact on the disease cycle of WSMV in North America because WCMs and virus are readily found in agroecosystems (Wegulo et al. 2008).

WSMV is in the family Potyviridae and the genus *Tritimovirus* and the virus is extremely variable. Three strains have been described: the Sidney 81 strain from Nebraska (Stenger et al 1998), the Type strain from Kansas, and the El Batán strain from the Central Highlands of Mexico (Choi et al. 2001). The Sidney 81 and Type strains are closely related to each other and to other isolates from the United States and Canada, while the El Batán strain is less closely related to either the Sidney 81 or the Type strain (Choi et al. 2001). Comparisons of isolates between the United States and those in central Europe and Russia indicate a distinct WSMV population in Eurasia (Rabenstein et al 2002).

Evolution of WSMV is thought to have occurred through genetic drift and negative selection (Choi et al. 2001). Although only three strains have been described, field studies conducted in Nebraska found 32 distinct restriction fragment length polymorphism (RFLP) isolates in five counties (McNeil et al. 1996). The majority of isolates could be grouped into three predominant genotypes. Surprisingly, there was greater variation within individual fields than among counties. Variation was found between years, demonstrating that frequencies of WSMV isolates co-circulate the region. This variability suggests WCMs are dispersing within and among fields and within counties, indicating WCMs may disperse over long distances. The high variation of

isolates discovered by McNeil et al. (1996) may also indicate WCMs disperse from a variety of alternate hosts containing different isolates of WSMV into the same field.

Plant viruses are associated with changes in chloroplast morphology and metabolism that cause chlorosis or necrosis (Goodman et al. 1986). Like many plant viruses, WSMV interferes with chloroplast development in systemically infected wheat (White and Brakke 1983, Brakke et al. 1988). Pigments, macromolecules, proteins, and nucleic acids are disrupted in chloroplasts, and chlorophyll is reduced in infected leaves (Brakke et al. 1988). WSMV causes a yellow to light green mosaic pattern of parallel discontinuous streaks on leaves (Watkins 2002, Wegulo et al. 2008). Recognizable symptoms of WSMV rarely occur in the fall; however, when late fall temperatures are unusually high, symptoms can be observed as early as the first week in October (Staples and Allington 1956). Symptoms generally appear with wheat growth in the spring as temperatures rise in April, and severity increases with continued warm weather (Staples and Allington 1956). Symptoms of plants that become infected in the fall during early growth stages, including stunting, rosetting and discoloration, are noticeable the following spring; however, spring infections generally lack these symptoms (Wegulo et al. 2008). Symptom expression is also affected by soil moisture, soil fertility, virus strain, and wheat variety (Staples and Allington 1956). Symptoms are often more severe at the edge of wheat fields or in patches near volunteer wheat (McMullen 2002).

Although WCMs cause damage to the plant by feeding upon it, the damage due to virus transmission is much more significant. A reduction in the size and weight of kernels and the number of kernels formed by infected plants contributes to yield loss

(Atkinson and Grant 1967). The severity and amount of yield loss due to WSMV infections is correlated with the time of infection, with earlier infections being more severe than late infections (McMullen 2002). Infections that occur by early tillering may cause growth to be restricted and few or no heads to emerge. Infections that occur in spring generally do not become severe or cause significant yield loss.

### **High Plains Virus**

Infection of wheat by High Plains virus (HPV) was discovered by Jensen et al. in 1994; however, the wide distribution in Texas, Nebraska, South Dakota, Idaho, Colorado, and New Mexico suggests it is endemic and may have been previously mistaken for WSMV. Symptoms of HPV are similar to those of WSMV with early infections causing small chlorotic spots that expand into a mosaic and general yellowing later on (Jensen et al. 1996). The WCM is responsible for vectoring HPV (Seifers et al. 1997) and western blot results in Nebraska showed rates of HPV on WCM infested wheat heads to be 46% over two years (Mahmood et al. 1998). Studies by Seifers et al. (2002) indicate that mites collected in Kansas, Montana, Nebraska, South Dakota and Texas differentially transmit HPV isolates and differential transmission appears to be related to variation in the ability of WCM colonies to transmit HPV rather than differences among HPV isolates. Mixed infections of WSMV and HPV are common (Mahmood et al 1998).

Two other viruses display symptoms nearly identical to HPV: wheat spot mosaic virus (Slykhuis 1956) and wheat spot chlorosis pathogen (Nault et al. 1970). Both wheat spot mosaic virus (WSpMV) and wheat spot chlorosis pathogen (WSCP) are transmitted by WCMs (Slykhuis 1956, Nault and Styer 1970). Both HPV and WSCP are unique in

that infected plants have double-membrane-bound ovoid bodies in the cytoplasm of the parenchyma, phloem, and epidermal cells (Jensen et al. 1996, Nault et al. 1970). All three viruses are similar in that attempts at leaf-rub inoculation have been unsuccessful (Slykhuis 1956, Nault et al. 1970, Skare et al. 2003) and all three viruses have a similar host range (Slykhuis 1956, Nault et al. 1970, Seifers et al. 1998). HPV, WSpMV and WSCP may in fact be the same virus; however, WSpMV-infected and WSCP-infected plant samples are no longer available for comparison studies (Skare et al. 2006).

### **Triticum Mosaic Virus**

Triticum mosaic virus (TriMV) is a new virus of wheat with WSMV-like symptoms discovered in Kansas in 2006 (Seifers et al. 2008). The WCM was found to be responsible for vectoring TriMV, and although initial studies indicated the number of wheat plants infected solely with TriMV was low, levels of infection of wheat in the field by both TriMV and WSMV were high (Seifers et al. 2009). Wheat infected with TriMV displays light-green or yellow streaking, spotting or mottling (De Wolf and Seifers 2008). TriMV has recently been isolated from 17% of symptomatic wheat samples in Colorado, Nebraska, Oklahoma, South Dakota, Texas and Wyoming (Burrows et al. 2008). In Nebraska, 27% of collected wheat samples displaying virus-like symptoms in 2008 were positive for TriMV (Burrows et al. 2008). The economic importance of TriMV is currently unknown (Seifers et al. 2008); however, plants infected with both TriMV and WSMV have more severe symptoms than single virus infections (Burrows et al. 2008) and mixed infections may cause plants to die prematurely or produce little or no grain (De Wolf and Seifers 2008).

### **Alternate Hosts of the WCM, WSMV, HPV and TriMV**

The WCM is able to survive on a variety of hosts other than wheat, including many native annual and perennial grasses (Connin 1956b). The WCM has been collected in the field from maize (*Zea mays* L.), (Sill and del Rosario 1959), sorghum (*Sorghum bicolor* (L.)), western wheat grass (*Agropyron smithii* Rydb.) (Gibson 1957), foxtail barley (*Hordeum jubatum* L.), and Canada wild rye (*Elymus Canadensis* L.) (Slykhuis 1955). Although wheat curl mites have been reported to survive on barley and rye, reproduction is diminished when compared to populations on wheat (Seifers et al. 1996, Seifers et al. 1997). A salivary phytotoxin of the WCM causes kernel red streaking (KRS) to occur on corn when the mite disperses from wheat (Nault et al. 1967, Liu et al. 2005); however, there is no evidence to suggest KRS causes yield reduction or loss of feeding value to corn (Nault et al. 1967, Tunac and Nagel 1969).

Alternate hosts that have been infected by WSMV include five oat varieties (McKinney 1949), barley, millet, *Panicum*, *Setaria*, and *Echinochloa* spp. (Sill and Agusiobo 1955, Brakke 1987); several grass species (del Rosario and Sill 1965, Christian and Willis 1993), sorghum (Harvey and Seifers 1991), and some varieties of maize (Brakke 1987).

Corn is susceptible to HPV and severe damage can result from infections to dent and sweet corn (Jensen et al. 1996). Early infection of susceptible corn causes stunting and pronounced chlorosis in young tissue, and as growth continues, chlorosis is amplified, with older tissue becoming reddened at the leaf margin (Jensen et al. 1996). In Texas, the incidence of HPV on corn is related to corn planting date and winter wheat



maturity. Corn planted 10 to 30 days after wheat heading has the highest incidence of infection (Fritts et al. 1999). Wheat curl mites that are actively dispersing as wheat matures are able to survive on corn and transmit HPV; however, corn planted 10 to 20 days before wheat heading may escape severe infections of HPV (Fritts et al. 1999) because corn plants will be more mature when mites begin to disperse and infect plants. Other hosts of HPV include cheat, barley, oat, rye, green foxtail, and yellow foxtail (Seifers et al. 1998).

Barley is susceptible to TriMV (Seifers et al. 2008); however, the effect or its significance is currently unknown. Other plant species infected by TriMV also remain unknown.

### **Management of the WCM**

The presence of pre-harvest volunteer wheat is important to the epidemiology of WSMV, HPV and TriMV. In order to survive over summer after harvest, the WCM needs an alternate host (Connin 1956a). In the central Great Plains, volunteer wheat is the most important host utilized by WCMs as a ‘green bridge’ between summer harvest and fall planting (Wegulo et al. 2008). Pre-harvest hail storms cause grain to be shattered on the ground and this leads to the production of volunteer wheat (Staples and Allington 1956). Volunteer wheat produced in this way germinates and grows quickly, and when wheat plants begin to dry back the WCM can disperse to new green plants (Staples and Allington 1956, Gibson and Painter 1956). Volunteer wheat plants provide a summer host for the WCM to survive on until wheat is planted in the fall. The later volunteer

wheat appears post harvest, the less likely a serious mite infestation will occur (Wegulo et al. 2008).

Destruction of pre-harvest volunteer wheat in the weeks before planting is the optimal strategy for controlling the WCM and preventing virus transmission; however, other tactics can also be used to reduce the risk of infection. Miticides have not provided efficient control of the WCM; however, cultural practices and resistant varieties have aided in management. Control strategies other than destruction of the 'green bridge' are often inadequate.

### **Chemical Control**

Pesticides have been of little consequence in controlling WCMs. Miticides are an inefficient means of control due to the habitat of the mite. The WCM causes wheat leaves to roll, and mites utilize the habitat within the leaf sheaths to provide them with protection from insecticidal exposure (Kantack and Knutson 1958). Carbofuran, a systemic insecticide, has been used in the fall at planting time to reduce the incidence of WSMV in the spring (Harvey et al. 1979).

### **Cultural Practices**

As mentioned previously, destruction of the 'green bridge' host is crucial to WCM management. To reduce WCM infestations in the fall and control WSMV, HPV and TriMV, destruction of pre-harvest volunteer wheat is essential (Staples and Allington 1956, De Wolf and Seifers 2008). Volunteer wheat should be destroyed at least two weeks before wheat is planted in the fall (Jiang et al. 2005, Wegulo et al. 2008). Paraquat

and glyphosate are herbicides that can be used to destroy the green-bridge host, effectively diminishing the ability of mites to survive between summer harvest and fall planting. Mite populations in volunteer wheat can be rapidly reduced when paraquat is applied and effects are apparent within a matter of days. Glyphosate also reduces mites in volunteer wheat; however, the effect is slower and glyphosate must be applied well before newly planted winter wheat emerges (Jiang et al. 2005). Tillage is efficient at reducing mite populations and results in a more rapid reduction when compared with glyphosate; however, tillage may increase erosion and soil moisture loss (Thomas et al. 2004). Another option is to blade immediately after harvest and again in late August or early September and apply atrazine with a spray boom mounted on the blade (Wegulo et al. 2008). Glyphosate is the most common choice for controlling pre-harvest volunteer wheat, and is preferred because of its broad-spectrum weed control, cost effectiveness, and probable lower toxicity to the applicator and other non-target animal and plant species (Jiang et al. 2005).

Volunteer wheat emerging in late summer, well after harvest, does not harbor the virus vector (Staples and Allington 1956). Although the WCM, WSMV and HPV are able to survive on several other hosts over the summer, and minor infections may occur from viruliferous mites derived from native grasses, the destruction of native perennial and annual grasses near wheat fields is not warranted as these grasses are not particularly important to the epidemiology of WSMV (Staples and Allington 1956, Orlob 1966b). Hosts other than pre-harvest volunteer wheat generally don't allow mites or virus to build up sufficiently to cause a severe epidemic, but rather serve as hosts for the long-term survival of mites and virus (Wegulo et al. 2008).

Another method of controlling the WCM and the viruses it transmits is adjusting the planting date of winter wheat. Typically, wheat planted later will be less exposed to mites in the fall (Staples and Allington 1956, Hesler et al. 2005, Wegulo et al. 2008). Mites and virus must survive on an alternate host between summer harvest and fall planting, and increasing the amount of time between harvest and planting may decrease the populations of mites that successfully survive and disperse to newly planted wheat. Mites that are able to colonize late-planted wheat will have less time to build up in population, disperse throughout the wheat field, and spread virus before cool weather begins. Temperatures must be considered when planting late, however. If temperatures in the fall and early winter remain warm, WCM populations can potentially build up, causing subsequent virus infections, no matter how late wheat is planted (Staples and Allington 1956). When wheat is planted near sources of mites, the later the crop is sown, the higher the yields will be (Slykhuis et al. 1957). However, if no virus source is nearby, late seeding may result in poor yields (Slykhuis et al. 1957).

### **Host Plant Resistance**

Mace (N02Y5117) is a hard red winter wheat cultivar released in 2007, primarily for its field resistance to WSMV and its adaptation to rain-fed and irrigated wheat production systems in Nebraska and adjacent areas in the northern Great Plains (Graybosch et al. 2009). Resistance to WSMV is conditioned by the *Wsm-1* gene. Previous lines derived from *Wsm-1* had poor bread-making or agronomic qualities; therefore, Mace is thought to be the world's first cultivar to carry the *Wsm-1* gene. During preliminary field trials with no presence of virus, Mace showed no significant

difference in yield when compared to Millenium, a moderately tolerant hard red winter wheat cultivar (Graybosch et al. 2009). Mace outperformed Millennium; however, when subjected to a uniform infection of WSMV (Graybosch et al. 2009). Mace yield was twice that of the highly susceptible cultivars Tomahawk and ‘Wesley’ when subjected to a uniform infection of WSMV. In Nebraska, Mace is recommended as a highly tolerant WSMV cultivar, while 2137, Millennium and Pronghorn have mild levels of resistance (Wegulo et al. 2008). Varieties resistant to WSMV have shown sensitivity to TriMV (Seifers et al. 2008), and due to its recent discovery, resistance to TriMV has not been investigated.

Temperature sensitive resistance to WSMV has been discovered in CO960293, KS03HW12, and cv. RonL wheat (Seifers et al. 2006, Seifers et al. 2007). RonL is a cultivar developed in Kansas that derived resistance from CO960293 and is best adapted for dryland production (Martin et al. 2007). When grown at 18°C under natural infestations of WCMs, CO960293 did not show any symptoms of WSMV, while wheat surrounding it became infected (Seifers et al. 2006). The same was true for KS03HW12 and cv. RonL when tested against six WSMV isolates (Seifers et al. 2007). These varieties did not perform well at 24°C, however. This type of resistance may be valuable where temperatures are cool following fall planting, but resistance will not hold up in warmer wheat growing regions (Seifers et al. 2006).

Resistant varieties that inhibit WCM reproduction effectively reduce WCM incidence. TAM 107, a WCM-resistant cultivar, provides resistance to WCMs and has been grown widely in western Kansas since 1988; however, the widespread popularity of

TAM 107 in Kansas and adjoining states has resulted in strains of the WCM that have adapted to the resistance gene (Harvey et al. 1995, Harvey et al. 1997). New varieties of wheat resistant to the WCM are being researched, but so far the results have not been as promising as desired, with varieties providing only moderate resistance or field tolerance (Somsen and Sill 1970, Harvey et al. 1999). Pubescence increases infestations of WCMs, subsequently increasing WSMV infection (Harvey and Martin 1980); however, less pubescent varieties are not currently available.

Collections of WCMs from Montana, Nebraska, South Dakota, Alberta, Canada and Kansas have been found to vary in the degree of virulence to different types of resistant wheat (Harvey et al. 1995, Harvey et al. 1999). Evidence exists that the WCM may consist of at least two separate lineages that may represent putative species (Carew et al. 2009). Wheat curl mites obtained in Nebraska, Montana, and Kansas were analyzed for genetic variability and it was determined that two types of WCM exist, type 1 (NE type) and type 2 (KS-MT-SD-TX) (Siriwetwawat 2006). Mixed populations were found in all three states, with type 1 dominant in Nebraska and type 2 dominant in Kansas and Montana. The majority of genetic variability of mites was found to be located within the wheat head, indicating the WCM has the ability to disperse at different scales.

It may be important, therefore, to include as many types of the WCM as possible in screening or breeding tests for resistance. Wheat curl mites utilized in resistance trials should be obtained from various locations because WCMs collected from a given location may not necessarily be representative of the broader region.

## Natural Enemies

Currently, there is no literature concerning natural enemies of the WCM; however, G.L. Hein (unpublished), has observed a correlation in field and greenhouse between thrips and WCM populations in western Nebraska. Mite populations in greenhouse colonies tended to drop rapidly when they became infested with thrips. It was observed that when populations of thrips in the field were high, mite density tended to be lower.

Mites are common prey for thrips throughout the temperate and tropical regions (Lewis 1973, Chazeau 1985), and some species of thrips traditionally thought of as pest species, such as the western flower thrips (*Frankliniella occidentalis* Pergande) have been reported to be predaceous and feed on mites and assist with mite control in some crops (Martin 2000, Sutherland 2006). Onion thrips (*Thrips tabaci* Lindeman) and the western flower thrips have been reported to feed on mite eggs (Chazeau 1985, Moritz et al. 2004).

Two species of thrips, *Haplothrips subtilissimus* Haliday and *Xylaplothrips fuliginosus* (Schille) have been found to be associated with a free-living gall mite species of the Eriophyoidea (Schliesske 1992). *Leptothrips mali* (Fitch), a predaceous thrips, is known to feed on the tomato russet mite, *Vasates lycopersici* (Masee), an eriophyid (Bailey and Keifer 1943, Anderson 1954). *L. mali* is one of the most common and widely distributed predaceous thrips in North America and has been collected in the majority of continental states (Bailey 1940). The presence of this species often indicates the presence of eriophyids that are difficult to see, and there is positive evidence of *L.*

*mali* feeding on eriophyids *Calepitrimerus baileyi* Keifer and *Eriophyes vitis* (Landois) (Bailey 1940).

The effect of thrips on the size of the host population depends on the density and reproductive potential of predator and prey. Predaceous thrips are often not as abundant as plant-feeders, thus the effect may be negligible (Lewis 1973, Ananthakrishnan 1984). Thrips also prefer a dry environment and do not do as well during moist conditions, such as a rainy spring (Sutherland 2006).

### **WCM Movement and Risk Prediction**

Because the movement patterns of WCMs directly affect the risk of WSMV developing, it is important to understand its dispersal mechanisms. WCMs have been reported to hitchhike on the legs of thrips and aphids (Connin and Staples 1957) and can walk between 4 and 5 cm per hour (del Rosario and Sill 1958); however, the most important method of dispersal available to the wheat curl mite is wind. Mites are protected from wind gusts within rolled wheat leaves and are not dislodged from plants accidentally by wind gusts, even when gusts approach 20-30 mph; rather, dispersal is actively initiated by adult behavior (Nault and Styer 1969). Wheat curl mites initiate dispersal by crawling to an exposed area of the leaf and holding their bodies perpendicular to the leaf surface, using the anal sucker for adherence. On heavily infested plants, swarms of WCMs can be seen on the tips of leaves. They form chains by crawling upon one another and attaching to each other with their anal suckers. Chains then break apart from the mite mass and disperse in air currents (Nault and Styer 1969).



There is literature concerning a migratory form of WCM known as a deutogyne, a larger, more robust mite able to move more actively about the plant (Somsen 1966). Migratory forms appear to increase in number more rapidly with diminishing food supplies. Deutogynes have the potential to disperse over larger areas; however, more research is needed to determine the conditions necessary for deutogyne development and the extent of increased dispersal. There has only been one report of deutogynes; therefore, the implications of this form are unknown.

Drying of the wheat head and flag leaf was previously thought to be the largest influence on WCM dispersal (Nault and Styer 1969). The WCM is not able to protect its body from desiccation, and therefore must colonize secluded areas of the host (Nault and Styer 1969). When plants begin to deteriorate, mite movement and dispersal to neighboring wheat increases (Hein 1999). Although plant deterioration may limit the ability of WCM populations to increase, the main influence on the level of dispersal is not related to plant condition but to the size of the source population (Thomas and Hein 2003). Volunteer wheat heavily infested with WCMs and WSMV is associated with an increased amount of WCM movement out of the source population and into nearby fields (Thomas and Hein 2003). Dispersal is also affected by temperature and light (Nault and Styer 1969). Dispersal of the wheat curl mite increases with both an increase in light and an increase in temperature.

In order to more efficiently control WCM and WSMV, growers must be able to predict the risk of an epidemic occurring in their fields. If WCMs are present in pre-harvest volunteer wheat in nearby fields, producers need to be able to predict the

potential of their field being infected. The ability to predict risk affords an opportunity for producers to utilize a more targeted approach in controlling volunteer wheat in risk areas, or if volunteer exists outside property lines, to utilize more resistant varieties or to delay planting to reduce risk. The incidence of WSMV is dependent on the distance the winter wheat field is removed from infested and infected volunteer wheat and the density of mite populations in the volunteer wheat (Staples and Allington 1956).

The first means of predicting WSMV spread by the WCM is to determine the density of WCMs present in the 'green bridge'. The most important green bridge host to the epidemiology of WSMV is pre-harvest volunteer wheat. Suitable temperatures and rainfall in summer and autumn can lead to a substantial 'green bridge' of volunteer wheat that influences WCM populations (Coutts et al. 2008a). Pre-harvest volunteer wheat provides suitable food and shelter for the wheat curl mite during summer months, allowing WCM populations to increase (Staples and Allington 1956).

Another method of predicting risk is the proximity and direction of the field to a significant green bridge host. Observations of natural infections of WSMV in Colorado indicate severity of WSMV decreases rapidly as the distance from the source of inoculum increases (Shahwan and Hill 1984). There is some evidence that wheat streak mosaic infection is more prevalent in the direction of the prevailing winds (Slykhuis 1955). Coutts et al. (2008a) showed that wind direction played a strong role in WSMV spread and observed a rapid decline of virus spread in crops upwind from the WSMV-infected field and a slow decline downwind from the WSMV-infected field. There is a significant

correlation between mite dispersal and wind velocity (Staples and Allington 1956); however, there is no evidence that suggests how far the WCM can move.

### **Remote Sensing**

Remote sensing is the science and art of collecting information about an object without physical contact (Jensen 2007). A remote sensor measures electromagnetic radiation emitted or reflected from an object or geographic area from a distance. Valuable information can be subsequently extracted from the data with mathematical and statistical based algorithms. Waves of electric and magnetic energy move together through space to form electromagnetic radiation, and this energy can be categorized by its frequency and wavelength. The electromagnetic spectrum of waves is divided into sections based on wavelength. From the shortest wavelength to the longest, the electromagnetic spectrum consists of gamma rays, x-rays, ultraviolet light, visible light, infrared, and radio waves.

### **Remote Sensing of Vegetation**

Vegetation has a unique spectral profile that is caused by variations in reflectance and absorbance at certain wavelengths of the electromagnetic spectrum. Energy that is intercepted by healthy green vegetation interacts with pigments, water and intercellular spaces (Jensen 2007). Reflectance in the visible portion of the electromagnetic spectrum (400-700 nm) is controlled by leaf pigments located in the chloroplasts. Strong absorption of energy required for photosynthesis causes low reflectance and transmittance in this region (Kumar et al. 2001). Most chloroplasts in plants contain chlorophyll (65%), carotenes (6%), and xanthophylls (29%); however, the percentage and

distribution of pigments is highly variable (Gates et al. 1965). Absorption peaks in the visible spectrum occur around 420, 490 and 660 nm, and these peaks are caused by strong absorption by chlorophyll (Kumar et al. 2001). For most green plants, absorbance in leaves is 80-95% in the blue region (400-500 nm), 60-80% in the green region (500-600 nm) and 80-90% in the red region (600-700 nm) (Loomis 1965).

There is a dramatic increase in reflectance of vegetation between the visible and the near-infrared (NIR) region of the electromagnetic spectrum. This area of increasing reflectance, from 670 to 780 nm, is known as the red edge. The 'red edge' is a unique feature in the spectral profile of vegetation that results from chlorophyll absorption in the red region of the spectrum, causing low reflectance in the red region, and high internal leaf scattering in the spongy mesophyll, that causes high reflectance in the NIR region (Horler et al. 1983b). These characteristics cause a sharp linear increase in reflectance between the red and the NIR region.

Reflectance in the NIR region is influenced by the volume of intercellular spaces in the spongy mesophyll (Gates et al. 1965) and the number of leaf layers with increased leaf layers, up to about seven layers, causing increased reflectance (Kumar et al. 2001). The absence of absorption by pigments causes strong reflectance and transmittance in this region (Clevers 1994). Leaves of most green plants only absorb about 5% of energy in the NIR region (700-1200 nm). The remaining energy is either reflected or transmitted (Loomis 1965). The high rate of reflectance and transmittance has evolved in plants to protect them from overheating (Gates et al. 1965). If energy in the NIR region were

absorbed with the efficiency of the visible region, plants would become too warm and proteins would become irreversibly denatured (Jensen 2007).

Reflectance in the mid-infrared region (1300-2500 nm) is characterized by strong water absorption in plant cells and reflectance is much lower than in the near-infrared region (Kumar et al. 2001). Vegetation that has greater turgidity has lower reflectance in the mid-infrared region and as moisture content decreases, reflectance increases significantly (Jensen 2007). The main water absorption bands are located at 1450 and 1960 nm (Clevers 1994).

### **Detecting Changes in Vegetation – Optical Responses to Plant Stress**

Remote sensing of vegetation can be utilized for a wide variety of applications including differentiating between species and phenological stages. Remote sensing has been utilized to detect changes in plant response caused by various stresses such as those due to moisture, nutrients, pests, and pathogens (Jones and Schofield 2008). Changes resulting from plant stress that may not be detected by sight or touch affect the amount and direction of radiation reflected and emitted from plants, and these changes can be detected through remote sensing (Jackson 1986).

Reflectance values in the electromagnetic spectrum are heavily dependent on the relative composition of all the pigments in the leaf including chlorophylls, carotenoids and flavonoids. Because pigments are important to leaf function, variations in pigment content, especially chlorophyll, are good indicators of stress (Sims and Gamon 2002, Jones and Schofield 2008). Early detection of plant stress relies on identification of spectral regions of reflectance most responsive to adverse growth conditions (Carter and

Miller 1994). Stress sufficient to inhibit chlorophyll production can be detected first as increased reflectance at wavelengths of weak absorption, between 690 and 700 nm (Carter 1993). Strong absorbing pigments must decrease dramatically in the violet-blue portion of the spectrum at 420 nm for reflectance to increase appreciably (Carter and Miller 1994).

Carter (1993) tested a variety of biological and physiochemical stresses on different plant species to determine differences in leaf reflectance response and the wavelengths of leaf reflectance most responsive to stress. The stresses tested were competition in loblolly pine, powdery mildew disease in golden euonymus, *Euonymus japonica* variety Aureo-marginata, senescence in live oak, exposure to herbicide (*N*, *N*-dimethylurea) in persimmon (*Diospyros virginiana* L.) increased atmospheric ozone in loblolly pine, the sandy soils and high salinity of a barrier island vs. a mainland site in slash pine, and short-term dehydration in switchcane (*Arundinaria tecta* [Walt.] Mull). Reflectance in the visible spectrum, particularly the green region near 550 nm and the red region near 710 nm, increased in response to stress, regardless of the agent of stress or the species of plant; however, the differences near 710 nm were greater than those near 550 nm. Infrared reflectance was more variable, with stress causing no change or inconsistent change. The increased reflectance near 700 nm represents a shift towards shorter wavelengths, or a 'blue-shift' of the red edge. A blue shift of the red edge is caused by decreased absorption in the red region due to decreased absorption by pigments, and increased absorption in the NIR region due to changes in cell structure or leaf layers. The response to stress in the visible spectrum was not unique for particular stress agents and

lends support to the idea that physiological responses to stress are similar across different types of stress.

### **The Red Edge of Reflectance**

Chlorophyll content in plants changes for a variety of reasons and these changes can be detected by observing wavelength shifts in the red edge of reflectance. Gates et al. (1965) was the first to note that changes in the red edge may be indicative of changes in chlorophyll content. It has been observed that as chlorophyll content increases, the red edge shifts to progressively longer wavelengths. Chlorophyll content generally increases as plants mature, and begins to decrease as plants undergo senescence (Baret et al. 1987). Collins (1978) observed that during plant growth and maturation, the red edge shifts progressively toward longer wavelength, and in wheat and grain sorghum, a very pronounced red shift occurs during the heading stage.

Baret et al. (1987) tested the effect of chlorophyll on the red edge by measuring the spectral behavior of vegetative wheat canopies and determined that the general behavior of the spectra was independent of cultivars and planting date, but strongly dependent upon the phenology of the canopy. Similar results were obtained by Boochs et al. (1990) and Railyan and Korobov (1993) for wheat canopies, with peak shifts of the red edge towards longer wavelengths until the stage of flowering, and a reversion during senescence until maturity.

The red edge shift is a unique phenomenon that can be observed independently of background variations and allows detection of subtle states of plant condition or stress (Collins 1978). Vegetation stress varies by type and degree, and may cause biochemical

changes at the cellular and leaf level that may influence pigment systems and canopy moisture content, or cause changes to the canopy structure, coverage or biomass (Clevers 1994). Chlorotic corn leaves have higher reflectance in the visible wavelengths, with the greatest effect at 540 nm, and a red edge shift towards shorter wavelengths when compared to healthy corn leaves (Horler et al. 1983a). In wheat, increasing chlorophyll concentrations causes a linear increase in the wavelength of the red edge towards longer wavelengths. Although increased leaf stacking causes higher reflectance in the NIR region, the red edge shift due to chlorophyll may be the most important single factor controlling reflectance (Horler et al. 1983a, Horler et al. 1983b).

Guyot et al. (1988) modeled reflectance for wheat leaves and wheat canopies to detect differences in the red edge. Reflectance of leaves displayed a shift toward longer wavelengths as chlorophyll concentration increased. When reflectance increased in the red region, or when reflectance decreased in the near infrared region, this position shifted towards the short wavelengths. Reflectance at the canopy level indicated that for green leaves with a high chlorophyll concentration, increasing leaf area index (LAI) caused the position of the red edge to shift towards longer wavelengths.

The position of the red edge is strongly dependent upon chlorophyll content and biomass or LAI, and an estimation of plant vitality may be possible by studying reflectance in the red edge (Boochs et al. 1990). Decreased vitality caused by abnormal or non-optimal environmental conditions may result in a decrease in leaf chlorophyll content (Clevers 1994). Munden et al. (1994) discovered a linear relationship between chlorophyll concentration and yield and an asymptotic relationship between the red edge



and chlorophyll concentration in winter wheat. Using these relationships, they were able to estimate chlorophyll concentration up to 0.5 mg/g and yield up to 6 t/ha with the red edge.

There is an indication that increases in reflectance and decreases in absorbance in the 695-725 nm wavelength range are general responses of leaf optics to plant stress and are highly consistent among varying stressors (Carter and Knapp 2001). Increased reflectance in the 400-850 nm wavelength range was observed to occur as a result of early infestation of mature loblolly pine, *Pinus taeda* L., by the southern pine beetle, *Dendroctonus frontalis* Zimm., and insufficient nitrogen fertilization in seedlings of radiate pine, *Pinus radiata* D. Don. These two stressors caused a shift of the red edge toward the blue end of the spectrum. The tendency of stressed leaves to lose chlorophyll and the absorption properties of chlorophyll explain the optical response to stress at the red-edge. Simulated in vitro examinations of chlorophyll concentrations on white fiberglass filters also displayed a shift of the red edge toward shorter wavelengths when chlorophyll was decreased (Carter and Knapp 2001).

It may also be possible to detect subtle spectral symptoms in the red edge that can serve as an early indicator of certain types of stress (Horler et al. 1983b, Rock et al. 1988). Remote sensing images of forests indicated a pre-visual 'blue-shift' of the red edge, or a shift towards shorter wavelengths, before damage occurred (Rock et al. 1988). Damage in forests was associated with a loss in total chlorophyll. Although the shift was noted, it was not statistically significant. This 'blue-shift' of the red edge can be quantified with a vegetation index.

## Vegetation Indices

Vegetation indices are transformations of spectral bands utilized to reduce the dimensionality of data, enhance sensitivity to plant biophysical parameters, compensate for atmospheric effects, and normalize topography, canopy or soil variations (Jensen 2007). Indices utilized to calculate the red edge of reflectance are based on the wavelength position of the transition between low reflectance in the red region of the spectrum and high reflectance in the NIR region (Horler et al. 1983a). Guyot and Baret (1988) developed a linear interpolation to determine the red edge wavelength by assuming the reflectance of the red edge could be simplified to a straight line centered on a midpoint between reflectance in the NIR region at 780 nm and the reflectance minimum of the chlorophyll absorption feature at 670 nm. This method of determining the red edge position (REP) uses only four wavelength bands. Theoretical studies using radiative transfer models determined that the position of the red edge as calculated with the REP index moves toward longer wavelengths as chlorophyll content increases, and the largest effects occur at low levels of chlorophyll (Clevers and Jongschaap 2001). The REP also moves towards longer wavelengths as LAI increases. When both chlorophyll content and LAI increase, the REP shifts towards longer wavelengths. The REP is fairly insensitive to soil background, and a small shift to longer wavelengths occurs with increasing soil reflectance. This shift is most pronounced at low LAI values, yet even at low LAI values, there is scarcely any influence on the position of the red edge. Simulation studies have also concluded that atmospheric conditions do not affect the position of the red edge. The red-edge index developed by Guyot and Baret (1988) offers a method to

measure changes in chlorophyll content and LAI without interference from external factors such as soil brightness and atmospheric conditions.

Heege et al. (2008) tested the REP index proposed by Guyot and Baret (1988) on the nitrogen status of winter wheat. Nitrogen increases chlorophyll concentration per unit area in the leaf; subsequently, reflectance is decreased in the visible spectrum. Nitrogen also increases vegetation fraction, causing increased reflectance in the NIR region. When the REP index was regressed against nitrogen status in wheat, the coefficient of determination ( $R^2$ ) was 0.970, the highest  $R^2$  of all vegetation indices tested.

Other mechanisms besides linear interpolation to calculate the red edge inflection point have been developed including an inverted Gaussian technique (Hare et al. 1986) and a three-point Lagrange interpolation technique (Dawson and Curran 1998). These two methods and the linear method (Guyot and Baret 1988) have been found to yield comparable results (Clevers and Jongschaap 2001). An approach locating the red edge position as the maximum first derivative of the reflectance in the region of the red edge using a third-order polynomial to fit the spectrum and computing the maximum of the derivative in the range of interest has also been developed (Demetriades-Shah et al. 1990); however, the technique is complex and computationally demanding (Baranoski and Rokne 2005). Baranoski and Rokne (2005) developed an unsupervised approach for determining the red edge with low, although variable, relative error across crops. This approach utilizes reflectance at fixed wavelengths, rather than supervising the choice of wavelengths.

Gitelson et al. (1996) developed a simple ratio to determine chlorophyll using the red edge. The ratio of reflectance at 750 nm to that near 700 nm was directly proportional to chlorophyll concentration in maple and horse chestnut leaves collected in spring, summer and autumn. Ratios of reflectance in the near infrared region to that near 550 nm were also sensitive to chlorophyll concentration in maple and horse chestnut leaves and could predict chlorophyll content with an error of less than 5 nmol/cm<sup>2</sup> (Gitelson and Merzlyak 1998).

Many methods exist for calculating the red edge, as well as for calculating a number of different vegetation indices. A vegetation index should maximize sensitivity to biophysical parameters of interest. To determine the best method to utilize, it is important to determine what variable the researcher is attempting to detect, and to compare the results of a variety of different vegetation indices with biophysical variables collected from the field.

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## CHAPTER 1

# Hyperspectral Remote Sensing to Detect Symptoms Associated with Wheat Curl Mite- Vectored Viruses

## Introduction

Wheat streak mosaic virus (WSMV) is an important pathogen of winter wheat in the western Great Plains and losses can range from negligible to complete crop failure. WSMV is vectored by the wheat curl mite, *Aceria tosichella* Keifer, and causes yellowing of the foliage and stunting and rosetting of plants infected in the fall at early growth stages (Watkins 2002, Wegulo et al. 2008). The wheat curl mite (WCM) is responsible for vectoring two other viruses to winter wheat that cause symptoms similar to WSMV: High Plains Virus (Seifers et al. 1997) and Triticum mosaic virus (Seifers et al. 2008); however, WSMV is the predominant WCM-vectored disease in Nebraska (Burrows et al. 2009).

The wheat curl mite is a microscopic eriophyid mite widely distributed in North America (Oldfield 1970). It can utilize a variety of hosts, including many native annual grasses (Connin 1956b), maize (Sill and del Rosario 1959), barley, and rye; however, its preferred host is winter wheat, *Triticum aestivum* L. (Seifers et al. 1996, Seifers et al. 1997). WCMs are typically located in protected areas on wheat plants (Kantack and Knutson 1954), and feeding causes curling of the edges of the wheat leaf and trapping of subsequent leaves (Slykhuis 1955, Somsen and Sill 1970). High populations of WCMs can cause yield loss from 1 to 15% (Harvey et al. 2000); however, the majority of damage results from viruses transmitted by the WCM.

Resistant plant varieties (Seifers et al. 2006, Seifers et al. 2007, Martin et al. 2007, Graybosch et al. 2009) and cultural controls (Slykhuis et al. 1957, Thomas et al. 2004, Hesler et al. 2005, Jiang et al. 2005) have been developed to manage the WCM; however,



the most important management strategy has been the control of pre-harvest volunteer wheat (Staples and Allington 1956). Pre-harvest volunteer wheat, primarily resulting from pre-harvest hail storms, provides a 'green bridge' host for mite survival and reproduction between wheat harvest and emergence of winter wheat in the fall (Gibson and Painter 1956, Staples and Allington 1956). Wheat curl mites disperse by wind and the risk of fall planted wheat becoming infected by virus increases with increasing proximity to pre-harvest volunteer wheat harboring the virus vector and the density of WCMs in the green-bridge host (Staples and Allington 1956, Coutts et al. 2008).

Although there are no efficient methods available to control the WCM or its vectored viruses once it enters winter wheat, an increased understanding of mite movement and subsequent virus spread can assist in determining the risk of serious virus infections in winter wheat. Growers that are aware of risk parameters associated with pre-harvest volunteer wheat can make better decisions regarding WCM management. The WCM is small and hard to sample and track; however, the viruses it vectors produce significant symptoms that can be visually detected.

Like many plant viruses, WSMV interferes with chloroplast development of systemically infected wheat (White and Brakke 1983, Brakke et al. 1988). Pigments, macromolecules, proteins, and nucleic acids are disrupted in chloroplasts and chlorophyll is reduced in infected leaves (Brakke et al. 1988). Physiological changes in plants that result from virus infection can be detected with remote sensing, often prior to visual symptoms.

Remote sensing has been utilized to detect changes in vegetation caused by various stresses such as those due to moisture (Liu et al. 2004, Clay et al. 2006), nutrients (Moges et al. 2004, Baret et al. 2007), pests (Pontius et al. 2008, Nansen et al. 2009), and pathogens (Nutter et al. 2002, Apan et al. 2004, Huang et al. 2004, Chen et al. 2007). Changes resulting from plant stress that cannot be detected by sight or touch may affect the amount and direction of radiation reflected and emitted from plants, and these changes may be detected through remote sensing (Jackson 1986).

Vegetation indices are often applied to remote sensing data to reduce the volume of data by taking advantage of characteristics of important spectral bands. Numerous vegetation indices have been developed and many are reported to be correlated with plant condition, stress, vegetation fraction, or chlorophyll content (Rouse et al. 1974, Vidal et al. 1994, Gitelson et al. 2002a, 2005,).

The objective of this study was to determine the effectiveness of using close-range hyperspectral data to detect reflectance characteristics in winter wheat that result from virus infection. To establish the effectiveness of these methods we determined the most useful spectral index for estimating changes in biophysical characteristics associated with virus symptoms and established the relationship between these biophysical variables and virus symptom development.

## Materials and Methods

**Study site.** Volunteer wheat was planted in two, 10-m x 10-m plots in the middle of a fallow wheat field at the University of Nebraska's High Plains Agricultural Laboratory near Sidney, Nebraska on 12 July, 2007 (41°14' 16" N, 103° 0' 4" W) and 21 July, 2008 (41°14' 21" N, 102° 59'57 " W) (Appendix A). Once wheat was established, plots were infested with WCMs obtained from pre-harvest volunteer wheat from other locations in western Nebraska. To infest plots with WCMs, locations of winter wheat that had suffered pre-harvest hail storms were identified and samples of pre-harvest volunteer wheat were collected from these locations to determine the WCM infestation level. When a good source population was identified, volunteer wheat was collected and used to infest simulated volunteer plots. The collected wheat infested with WCMs was manually spread over the growing volunteer source plots. To create a variable infection rate, the amount of biomass spread over source plots was variable. Two plots were infested on 14 August, 2007 and two plots were infested on 26 August, 2008.

A variety of wheat susceptible to WSMV was planted surrounding the volunteer plots in the fall. Millenium was planted around source plots on 8 September, 2007 (12.67 ha), and Overland was planted around source plots on 16 September, 2008 (8.82 ha). A uniform grid of sampling points consisting of concentric circles was established around each plot. Sampling points were established and flagged at 7.6 m increments in each cardinal direction from the center of each plot and in lines 45° from the four cardinal directions. Thus, eight sampling points made up a ring at each 7.6 m increment from the center of the volunteer plot. Additional intermediate sampling points were added

between the eight sampling points in each ring to fill out the sampling scheme. In 2007-08, two intermediate sampling rings were added between the sampling rings at 7.6 m and 15.2 m and 15.2 m and 22.9 m to more effectively capture the gradient of virus symptoms. In 2007-08, seven rings were located between 7.62 and 38.1 m from the center of the north source plot (n=59) and eight rings were located between 7.6 and 45.7 m from the center of the south plot (n=69). Due to serious weed infestations present across much of one plot area, only one plot was utilized in 2008-09, and eight sampling rings were located between 7.6 and 61.0 m from the center of the source plot (n=76).

**Biophysical measurements.** When virus symptoms appeared in the spring, measurements of relative chlorophyll content, leaf area index (LAI), and virus incidence were taken. The majority of relative chlorophyll content and LAI measurements were taken between 3 June and 6 June, 2008 and between 5 June and 6 June, 2009. Relative chlorophyll content was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd. Osaka, Japan). At each sampling location, readings were taken from twenty randomly selected flag leaves and averaged. Readings were taken at approximately one-third the distance from the stem to the end of each flag leaf. Leaf area index, the ratio of foliage area to the ground, was determined at each sampling location with the LAI-2000 Plant Canopy Analyzer (Li-Cor, Inc., Lincoln, NE). LAI was determined by one above canopy and four below canopy readings made along a diagonal transect between rows.

After relative chlorophyll content and LAI were determined, ten tillers were randomly selected within one meter of each sampling location, bagged, returned to the

laboratory, and frozen until virus infection could be determined. Plant samples were collected between 4 June and 6 June, 2008 and on 23 June, 2009. Virus incidence in each tiller was determined using enzyme-linked immunosorbent assay (ELISA) for WSMV (Seifers et al. 1997, Seifers et al. 2002).

Samples were prepared by extracting plant sap from approximately 0.2 g of frozen flag leaf tissue from the flag leaf with a leaf press in 1.3 mL coating buffer (pH 9.6; 1.59 g  $\text{Na}_2\text{CO}_3$ , 2.93 g  $\text{NaHCO}_3$ , brought to one liter with distilled water). Two plant samples, known to be positive for WSMV, were used in each plate to verify the effectiveness of the ELISA run. Two control samples from healthy wheat were also used in each plate. For each sample, 200  $\mu\text{L}$  of plant extract were added to each of two sample wells of an ELISA plate (flat bottom 96-well plates, Immulon, Dynex Technologies, Chantilly, VA). Plates were incubated at 37°C for one hour then rinsed three times (1 minute/rinse) in PBS wash (pH 7.4; phosphate-buffered saline [PBS] and 0.05% Tween 20). Next, 200  $\mu\text{L}$  of WSMV antiserum (diluted 1:4000) were added to each sample well, and plates were incubated for one hour at 37°C then rinsed as described above. Following the rinses, 250  $\mu\text{L}$  of blocking buffer in 1X PBS (5.0 g nonfat dry milk; 10  $\mu\text{L}$  antifoam A; brought to 100 mL with 1X PBS) were added to each well, incubated for one hour at 37°C and rinsed as described above. Next, 200  $\mu\text{L}$  goat anti-rabbit IgG-alkaline phosphatase label, diluted 1:3000 in dilution buffer (pH 7.4; 0.5 mL Tween 20, 20 g PVP, 2.0 g Ovalbumin, 0.2 g sodium azide, brought to one liter with distilled water), were added to each well followed by one hour incubation at 37°C and rinsed as described above. Finally, 200  $\mu\text{L}$  phosphatase substrate (0.714 mg/mL) were added to each well and incubated at room temperature for one hour.

Absorbance of each ELISA plate was read at 405 nm (MR 4000 Micro ELISA plate reader; Dynatech Laboratories, Chantilly, VA). Plants were considered positive for WSMV when the mean absorbance of the replicate samples was greater than two times the mean absorbance of negative controls in the sample plate.

In 2008, the majority of relative chlorophyll content, LAI and ELISA samples were obtained within three days of each other; however, LAI readings taken in the north plot were delayed by approximately two weeks due to unfavorable weather conditions. In 2009, the majority of relative chlorophyll, LAI and ELISA samples were taken within four days of each other; however, a small number of LAI readings were delayed by one week due to unfavorable weather.

**Hyperspectral measurements.** Reflectance data were obtained approximately 1.5 m above the plant canopy, between 1000 and 1400 hours CDT, at each sampling location using two Ocean Optics 2000 hyperspectral field radiometers (one to measure downwelling irradiance and one to measure upwelling radiance) fitted with a 25° field of view fiber optic. The radiometers and collecting fibers were mounted on a sensor pole approximately 2.4 m in length (Appendix A). The serial ports on the Ocean Optics were connected with a self-contained backpack that included: 1) a WiFi router for the computer connection, 2) a serial terminal server to convert the radiometer serial connection to a TCP/IP network connection, 3) a 12 volt gel cell battery, and 4) a voltage converter to provide power to the radiometers (Appendix A). A computer utilized the WiFi connection to download reflectance data.

The radiometer was calibrated before each data-collection session using a Lambertian reflectance panel with a nominal reflectance of 99%. The Ocean Optics 2000 measures 2,023 wavelength bands that range from 350-1,025 nm and has a sampling interval of 0.38 nm. Reflectance data were collected on 3 June (north plot), and 4 June (south plot), 2008 and on 5 June, 2009. Reflectance collection occurred within a 3-4 day window of obtaining the majority of ground truth data in both years. Hyperspectral measurements were taken prior to removing samples to test for virus infection rates.

The spectral reflectance of samples that had virus infection rates of 100% (n=8), 50% (n=6), or 0% (n=12) in 2008-09 according to ELISA results were averaged and plotted against wavelength to determine differences in the spectral profiles. To reduce the volume of data and identify important spectral bands, ten vegetation indices (Table 1.1) were calculated from the hyperspectral data. Vegetation indices utilized have been reported in the literature to be correlated with plant condition, chlorophyll content, vegetation fraction, or stress.

**Statistical analysis.** The correlation between the biophysical variables was determined for each data set with PROC CORR (SAS Institute 2002-2003). Linear regression was determined using PROC REG (SAS Institute 2002-2003) to compare the coefficient of determination ( $R^2$ ) for each spectral index against each biophysical variable for the three data sets. The slopes and intercepts of the regression lines for each biophysical variable were compared with PROC GLM (SAS Institute 2002-2003).

## Results

The sampling pattern utilized in this study was designed to obtain a gradient of virus symptoms. A gradient of virus symptoms was observed each spring, with symptom severity decreasing with distance from the WCM source plot (Appendix A). Reflectance spectra of Ocean Optics ‘raw’ bands showed that wheat at sampling locations infected with WSMV exhibited differences in spectral reflectance that could be discriminated from non-diseased sampling locations. The average spectral profiles of wheat samples that had 100%, 50%, or 0% virus incidence according to ELISA testing in the 2008-09 data differed across wavelengths (Fig 1.1). The most sensitive spectral reflectance range, determined by visual observation, was located in the near infrared region (NIR; 760-925 nm). This was followed by selected ranges in the red (680 nm) and green (550 nm) regions. Virus infected samples had higher reflectance values than uninfected samples in the blue, green, and red regions of the spectrum. The reverse was true for the NIR region where virus infected samples had lower reflectance than uninfected samples.

The correlation between the biophysical variables was significant in all cases except the correlation between LAI and percent virus incidence in the 2007-08 south data set (Table. 1.2). Significant correlation coefficients ranged between -0.56 and -0.70 for relative chlorophyll content and percent virus incidence, between 0.34 and 0.68 for relative chlorophyll content and LAI, and between -0.59 and -0.70 for LAI index and percent virus.

All regression models were significant when relative chlorophyll content was regressed on each vegetation index (see Tables 1.3, 1.4, 1.5). The coefficients of



determination for relative chlorophyll content regressed on the spectral indices ranged from 0.13 to 0.73. The REP index produced the highest  $R^2$  with relative chlorophyll content over all three data sets. Although other spectral indices (PRI, NDVI, Chl, SI,  $Cl_{red\ edge}$ , D-chl-ab, and GNDVI) had high  $R^2$  when regressed on relative chlorophyll content in one or two data sets, the  $R^2$  were not as consistent as REP over all three data sets.

All regression models were significant when LAI was regressed on each vegetation index (see Tables 1.3, 1.4, and 1.5). The  $R^2$  for LAI regressed on the spectral indices ranged from 0.43 to 0.72 in the 2007-08 north and 2008-09 plots. Lower values were seen for the 2007-08 south plot (range: 0.19-0.38). The highest  $R^2$  for LAI were PRI,  $Cl_{red\ edge}$  and D-chl-ab for the 2007-08 north plot,  $Cl_{red\ edge}$  for the 2007-08 south plot, and REP for the 2008-09 plot.

All regression models were significant when percent virus infection was regressed on each vegetation index in all data sets except VF and VARI for the 2007-08 south data set (see Tables 1.3, 1.4, and 1.5). Significant regression models of percent virus infection with the spectral indices produced coefficients of determination ranging from 0.10 to 0.53. The highest  $R^2$  were for REP over all three data sets.

Overall, the REP index had the highest  $R^2$  when regressed against the majority of biophysical variables (Table 1.6). The slopes and intercepts of the regression lines for relative chlorophyll content versus the REP index for each plot were not significantly different. The  $R^2$  for the combined models was 0.63 ( $P < 0.0001$ ;  $n = 188$ ; Fig. 1.2). The intercepts of the regression lines for LAI against the REP index were not significantly

different between the 2007-08 north plot and the 2008-09 plot; however, they were significantly different from the 2007-08 south plot (F value=4.89; df=1,198; P=0.0281).

The slopes of the regression between LAI and REP were not significantly different between the 2007-08 north plot and the 2008-09 plot; however, they were significantly different from the 2007-08 south plot (F=4.82; df=1,198; P=0.0293). Combining the 2007-08 north and 2008-09 plot resulted in a  $R^2$  of 0.64 ( $P<0.0001$ ; n=132; Fig. 1.3).

The slopes and intercepts of the regression lines for percent virus incidence against the REP index for each plot were not significantly different. The combined models yielded an  $R^2$  of 0.41 ( $P<0.0001$ ; n=202; Fig. 1.4).

## Discussion

Vegetation stress varies by type and degree, and may cause biochemical changes at the cellular and leaf level that may influence the concentration of pigments in the leaf or cause changes to the canopy structure, coverage or biomass (Clevers 1994).

Reflectance values in the electromagnetic spectrum are heavily dependent on the relative composition of all the pigments in the leaf including chlorophylls, carotenoids and flavonoids, because pigments are important to leaf function, and variations in pigment content, especially chlorophyll, are good indicators of stress (Sims and Gamon 2002, Jones and Schofield 2008). Detection of plant stress relies on identification of spectral regions of reflectance most responsive to adverse growth conditions (Carter and Miller 1994).

Gates et al. (1965) was the first to note that changes in the red edge (670-780 nm) may be indicative of changes in chlorophyll content. It has been observed that as chlorophyll content increases, the red edge shifts to progressively longer wavelengths. Measurements at the canopy level indicate that for green leaves with high chlorophyll concentration, increasing LAI causes the position of the red edge to shift towards longer wavelengths (Guyot et al. 1988). A decrease in chlorophyll content or LAI results in a shift of the red edge toward shorter wavelengths. This shift is known as a blue-shift of the red edge.

Averaged reflectance spectra of Ocean Optics raw bands displayed a blue-shift of the red edge in plants infected with WSMV. There was a pronounced blue-shift of the red edge for sample points that had 100% infection compared to uninfected sampling

points. When 50% of plants at sampling locations were infected with WSMV, there was a blue-shift of the red edge when compared to uninfected samples; however, the shift was less pronounced than for 100% infected sampling points. These observations indicate WCM-vectored viruses have an effect on the red edge of reflectance.

Guyot and Baret (1988) developed the REP index to detect changes in the red edge of reflectance. The REP index utilizes only four wavelength bands. The REP index has been reported to detect changes in chlorophyll content and LAI, symptoms associated with WCM-vectored viruses, and that REP values move towards longer wavelengths as chlorophyll content and LAI increase (Clevers and Jongschaap 2001). This index is also reported to be fairly insensitive to soil background and not affected by atmospheric conditions (Guyot and Baret 1988).

The REP index had a significant relationship with chlorophyll over the three data sets with the  $R^2$  ranging between 0.62 and 0.71. The REP index also had a significant relationship with LAI over all three data sets; however, the  $R^2$  was much lower for the 2007-08 south plot ( $R^2=0.27$ ) when compared to the other two data sets (2007-08 north:  $R^2=0.62$ ; 2008-09:  $R^2=0.72$ ). Mean LAI measurements in the 2007-08 south data set ranged between 1.7 and 3.9 while mean LAI measurements had a larger range in the 2007-08 north (range: 0.97-4.07) and 2008-09 (range: 0.76-4.16) data sets. Mean LAI values were not significantly different between the 2007-08 north (mean: 2.00) and 2008-09 data sets (mean: 2.09); however, they were significantly lower than mean LAI values for the 2007-08 south data set (mean: 2.72) according to the Student-Newman-Keuls test. Visible virus symptoms in the field surrounding the 2007-08 source plot were lower than

the other two data sets. Because virus symptoms were less severe, an adequate gradient of LAI values may not have been obtained, resulting in a low correlation between REP and LAI.

The REP index had a significant relationship with percent virus infection for all three data sets. The coefficients of determination for percent virus infection ranged from 0.23 to 0.53, lower than those associated with chlorophyll in all three data sets and LAI in the 2007-08 north and 2008-09 data sets. The REP index may have a better relationship with chlorophyll and LAI than percent virus infection due to sample size. More samples were utilized to determine chlorophyll content and LAI at each sampling point than were used to determine percent virus infection. Chlorophyll content was measured on 20 randomly selected flag leaves within a one meter area. LAI values were obtained by measuring biomass with a field of view equal to three times the canopy height. Virus infection, however, was only obtained for ten tillers within one meter of each sampling point. The smaller sample size utilized for percent virus infection may have resulted in greater variance in virus infection rate.

The accuracy in measuring percent virus infection was less than that for chlorophyll and LAI. Relative chlorophyll and LAI measurements were measured with instruments that provide continuous readings; however, virus infection rate was estimated only to the nearest 10%. Increasing the number of samples tested for WSMV by ELISA may have improved the ability to detect smaller differences in virus infection rates between sampling locations; however, increasing sample size was not feasible due to time and cost constraints.

Virus titer may also have affected the relationship between percent virus infection and the REP index. When plants become severely infected, the amount of green tissue decreases substantially. Virus titer may decrease with decreasing green leaf tissue, reducing the ability for ELISA to detect WSMV in severely infected plants.

The correlation between biophysical variables was significant in all instances except for the correlation between LAI and percent virus incidence in the 2007-08 south data set. Lack of a significant correlation and low correlation between LAI values in the 2007-08 south plot and the other biophysical variables can be attributed to the inability to obtain an adequate gradient of LAI values. The significant and high correlation between the biophysical variables indicates there is a relationship between relative chlorophyll content, LAI and percent virus infection. As percent virus infection increased, both relative chlorophyll content and LAI decreased, and these variables can be detected with remote sensing.

Detectable changes in REP values reported in the literature are those that are most strongly associated with WCM-vectored viruses. Although other vegetation indices showed strong relationships with the biophysical variables tested, REP was the most consistent vegetation index in predicting relative chlorophyll content, LAI, and percent virus infection.

For proximal data, the REP index can be successfully utilized to detect WCM-vectored viruses. REP may assist in future studies that aim to detect the virus from aerial platforms. Several aerial hyperspectral imagers are available, including the Airborne Imaging Spectroradiometer (AISA Eagle) (Spectral Imaging, Ltd, Oulu, Finland), the

Compact Airborne Spectrographic Imager (CASI) (ITRES Research, Ltd, Calgary, Alberta, Canada), and the Airborne Visible-Infrared Imaging Spectrometer (AVIRIS) (NASA JPL, Pasadena, CA). The REP index has the potential to be applied to aerial remote sensing data to quantify the spatial spread of virus symptoms from a source WCM population. As previously mentioned, an understanding of mite movement and virus spread can assist in determining the risk of mite movement into fall-planted wheat. An understanding of the spatial spread of virus symptoms can be used to develop risk parameters of mite movement from WCM source populations, and risk zones can be utilized by wheat growers to make better decisions regarding WCM management.

The REP index may also be useful to crop insurance adjusters to quantify damages resulting from WCM-vectored viruses. Only four wavelength bands are necessary to calculate the REP index. A sensor that collects reflectance values at the four bands used to calculate the REP index could be developed and utilized to determine the spatial spread and impact of virus in wheat fields, decreasing the amount of time spent surveying fields and increasing the ability to accurately determine the area affected by virus.

It may also be possible to utilize remote sensing satellites to determine the percent of wheat infected with virus over large wheat growing areas. Hyperion is a hyperspectral remote sensor on NASA's Earth Observing-1 (EO-1) that acquires imagery in 220 spectral bands (400-2500 nm) with a 10 nm band width. Imagery is acquired at 30 meter spatial resolution, and this type of imagery has the potential to be used for extensive virus

surveys in wheat-growing regions of Nebraska to determine the yearly impact of WCM-vectored viruses.



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## Tables

Table 1.1. Vegetation indices used in this study.

Vegetation Index	Equation	Reference
Physiological Reflectance Index (PRI)	$PRI = \frac{\rho_{531} - \rho_{570}}{\rho_{531} + \rho_{570}}$	Gamon et al. 1992
Normalized Difference Vegetation Index (NDVI)	$NDVI = \frac{\rho_{nir} - \rho_{red}}{\rho_{nir} + \rho_{red}}$	Rouse et al. 1974
Stress Index	$SI = \frac{\rho_{693}}{\rho_{759}}$	Vidal et al. 1994
Chlorophyll Index (ChI)	$ChI = \frac{\rho_{770}}{\rho_{710}}$	Gitelson et al. 2005
Vegetation Fraction (VF)	$VF = \frac{\rho_{550} - \rho_{670}}{\rho_{550} + \rho_{670}}$	Gitelson et al. 2002a
Chlorophyll Red Edge (Cl <sub>red edge</sub> )	$Cl_{rededge} = \frac{\rho_{NIR}}{\rho_{rededge}} - 1$	Gitelson et al. 2005
Visible Atmospherically Resistant Index (VARI)	$VARI = \frac{\rho_{green} - \rho_{red}}{\rho_{green} + \rho_{red} + \rho_{blue}}$	Gitelson et al. 2002b
D-chl-ab	$D - chl - ab = \frac{(\rho_{760} - \rho_{740}) / 2}{\rho_{560}}$	Gitelson and Merzlyak 1996
Green Normalized Difference Vegetation Index (GNDVI)	$GNDVI = \frac{\rho_{NIR} - \rho_{green}}{\rho_{NIR} + \rho_{green}}$	Gitelson et al. 1996
Red-edge Position (REP)	$REP = 700 + 40 \frac{\rho_{(rededge)} - \rho_{(700nm)}}{\rho_{(740nm)} - \rho_{(700nm)}}$	Guyot and Baret 1988 Guyot et al. 1988
	$\rho_{rededge} = \frac{\rho_{670} + \rho_{780}}{2}$	Clevers 1994

Table 1.2. Correlation between biophysical variables associated with virus symptoms<sup>\*</sup>.

	Correlation Coefficient		
	2007-08 North	2007-08 South	2008-09
Relative Chlorophyll vs. % Virus Infection	-0.69 <sup>*</sup>	-0.56 <sup>*</sup>	-0.70 <sup>*</sup>
Relative Chlorophyll v. Leaf Area Index	0.60 <sup>*</sup>	0.34 <sup>*</sup>	0.68 <sup>*</sup>
Leaf Area Index vs. % Virus Infection	-0.59 <sup>*</sup>	-0.20	-0.70 <sup>*</sup>

<sup>\*</sup>Significant at  $p < 0.005$ .



Table 1.3. Coefficients of determination ( $R^2$ ) for vegetation indices regressed on biophysical variables for the north plot in the 2007-08 growing season\*.

Vegetation Index <sup>a</sup>	Chlorophyll n=59	LAI <sup>b</sup> n=58	% Virus Infection n=59
PRI <sup>c</sup>	0.54	<b>0.66</b>	0.42
NDVI	0.48	0.59	0.24
Chl	0.50	0.65	0.33
SI	0.56	0.61	0.30
VF	0.26	0.52	0.13
Cl <sub>red edge</sub>	0.54	<b>0.66</b>	0.38
VARI	0.27	0.52	0.14
D-chl-ab	0.49	<b>0.66</b>	0.40
GNDVI	0.53	0.60	0.28
REP	<b>0.71</b>	0.62	<b>0.44</b>

\* All values significant at  $p < 0.01$ .

<sup>a</sup>Physiological reflectance index (PRI); normalized difference vegetation index (NDVI); stress index (SI); chlorophyll index (Chl); vegetation fraction (VF); chlorophyll red edge (Cl<sub>red edge</sub>); visible atmospherically resistant index (VARI); green normalized difference vegetation index (GNDVI); red edge position (REP).

<sup>b</sup>Leaf area index (LAI).

<sup>c</sup>Coefficients of determination in bold are the highest of the vegetation indices tested for each biophysical variable.

Table 1.4. Coefficients of determination ( $R^2$ ) for vegetation indices regressed against biophysical variables for the south plot in the 2007-08 growing season\*.

Vegetation Index <sup>a</sup>	Chlorophyll	LAI <sup>b</sup>	% Virus Infection
	n=69	n=69	n=69
PRI <sup>c</sup>	0.44*	0.19*	0.20*
NDVI	0.26*	0.27*	0.11*
Chl	0.31*	0.28*	0.10*
SI	0.35*	0.31*	0.14*
VF	0.13*	0.25*	0.04
CI <sub>red edge</sub>	0.49*	<b>0.38*</b>	0.19*
VARI	0.15*	0.27*	0.05
D-chl-ab	0.36*	0.34*	0.14*
GNDVI	0.16*	0.23*	0.13*
REP	<b>0.62*</b>	0.27*	<b>0.23*</b>

\*Significant at  $p < 0.01$ .

<sup>a</sup>Physiological reflectance index (PRI); normalized difference vegetation index (NDVI); stress index (SI); chlorophyll index (Chl); vegetation fraction (VF); chlorophyll red edge (CI<sub>red edge</sub>); visible atmospherically resistant index (VARI); green normalized difference vegetation index (GNDVI); red edge position (REP).

<sup>b</sup>Leaf area index (LAI).

<sup>c</sup>Coefficients of determination in bold are the highest of the vegetation indices tested for each biophysical variable.

Table 1.5. Coefficients of determination ( $R^2$ ) for vegetation indices regressed on biophysical variables in the 2008-09 growing season\*.

Vegetation Index <sup>a</sup>	Chlorophyll	LAI <sup>b</sup>	% Virus Infection
	n=60	n=75	n=74
PRI <sup>c</sup>	0.64	0.58	0.45
NDVI	0.66	0.57	0.43
Chl	0.58	0.65	0.43
SI	0.70	0.57	0.42
VF	0.43	0.43	0.27
Cl <sub>red edge</sub>	0.71	0.64	0.49
VARI	0.44	0.44	0.28
D-chl-ab	0.49	0.62	0.40
GNDVI	0.67	0.61	0.47
REP	<b>0.73</b>	<b>0.72</b>	<b>0.53</b>

\* All values significant at  $p < 0.0001$ .

<sup>a</sup>Physiological reflectance index (PRI); normalized difference vegetation index (NDVI); stress index (SI); chlorophyll index (Chl); vegetation fraction (VF); chlorophyll red edge (Cl<sub>red edge</sub>); visible atmospherically resistant index (VARI); green normalized difference vegetation index (GNDVI); red edge position (REP).

<sup>b</sup>Leaf area index (LAI).

<sup>c</sup>Coefficients of determination in bold are the highest of the vegetation indices tested for each biophysical variable.

Table 1.6. Regression models for red edge position (REP) regressed on biophysical variables for each data set\*.

	Chlorophyll	LAI	% Virus Infection
<b>2007-08</b>			
<b>North Plot</b>	SPAD=1.95*REP-1369.98 R <sup>2</sup> =0.71; n=59	LAI=0.20*REP-139.54 R <sup>2</sup> =0.62; n=58	%=10.35*REP+7517.32 R <sup>2</sup> =0.44; n=59
<b>2007-08</b>			
<b>South Plot</b>	SPAD=1.82*REP-1279.39 R <sup>2</sup> =0.62; n=69	LAI=0.14*REP-100.25 R <sup>2</sup> =0.27; n=69	%=-7.56*REP+5508.98 R <sup>2</sup> =0.23; n=69
<b>2008-09</b>			
	SPAD=1.68*REP-1175.20 R <sup>2</sup> =0.73; n=60	LAI=0.22*REP-158.35 R <sup>2</sup> =0.72; n=75	%=-10.09*REP+7329.82 R <sup>2</sup> =0.53; n=75

\*All values significant at p<0.0001.

## Figures

Fig. 1.1. Reflectance spectra of samples containing various wheat streak mosaic virus (WSMV) infection levels: (a) mean reflectance for samples with 100%, 50%, and 0% virus and (b) difference of means between samples with 100% WSMV and without WSMV.

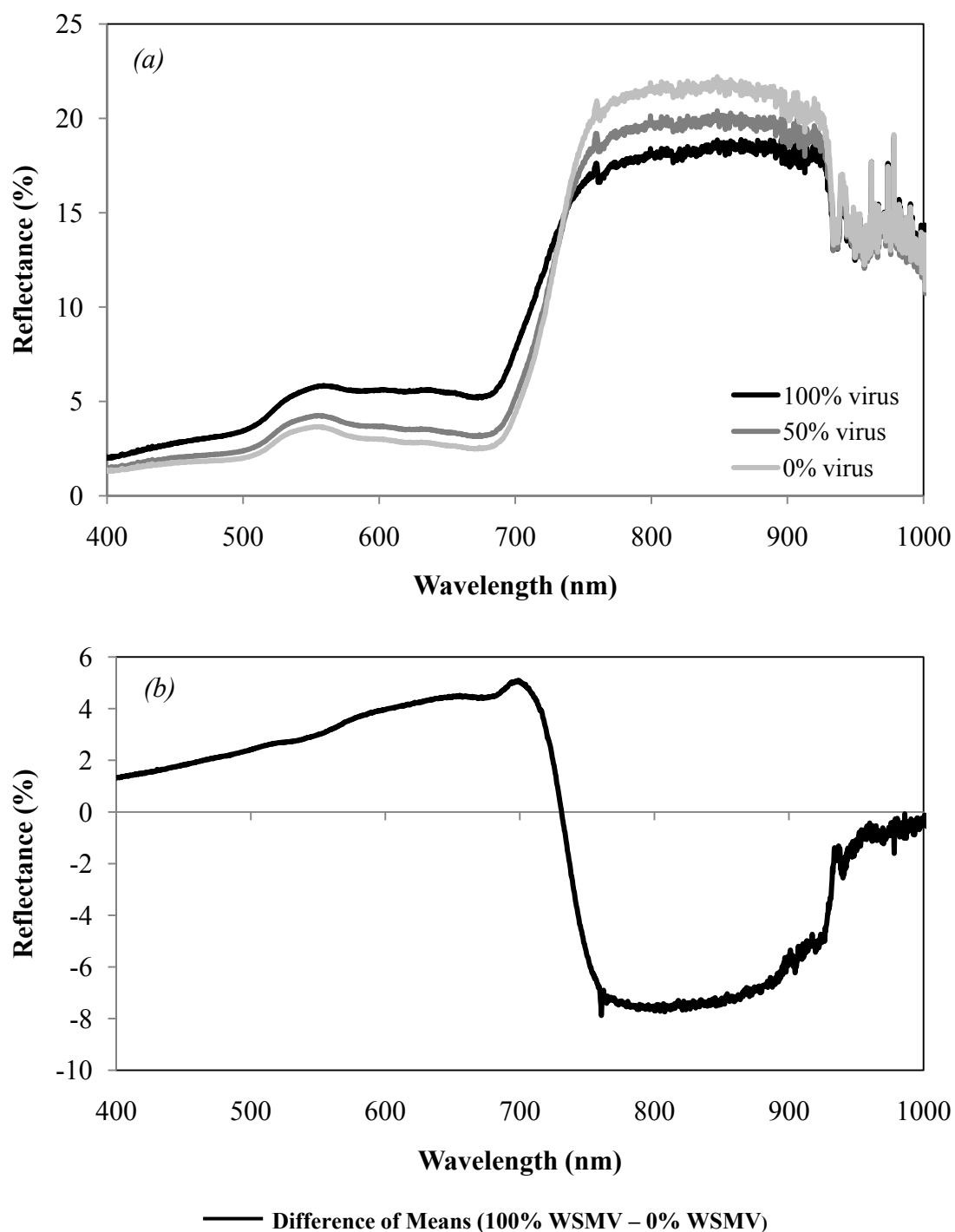


Fig. 1.2. Relationship between relative chlorophyll content and the red edge position (REP). The regression lines of the relative chlorophyll content vs. REP relationship for each data set (black lines) and all data combined (solid red line) are shown. The regression equation, the coefficient of determination ( $R^2$ ), and the number of samples (n) for all data combined are shown.

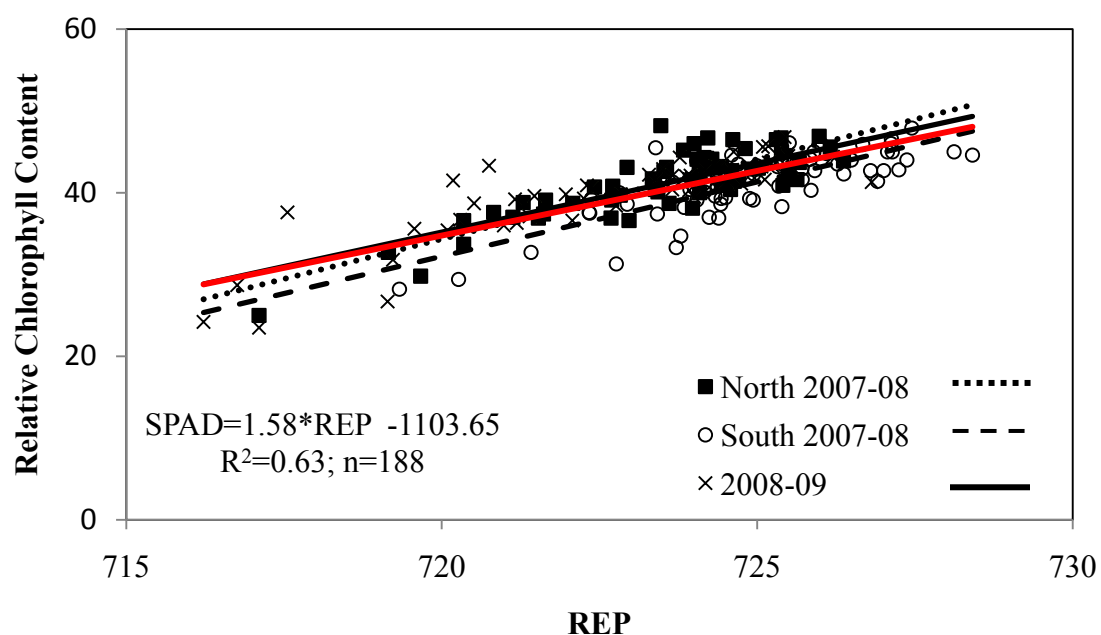


Fig. 1.3. Relationship between leaf area index (LAI) and the red edge position (REP). The regression lines of the LAI vs. REP relationship for each data set (black lines) and the regression line for the 2007-08 North and 2008-09 plot combined (red line) are shown. The regression equation, the coefficient of determination ( $R^2$ ), and the number of samples (n) for the combined data sets are shown.

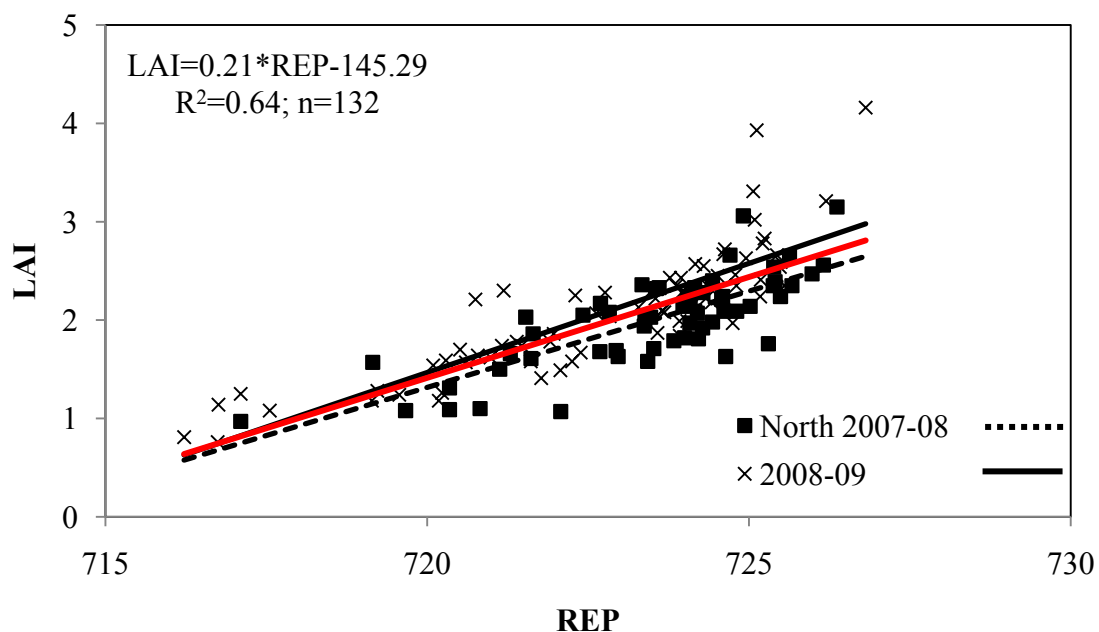
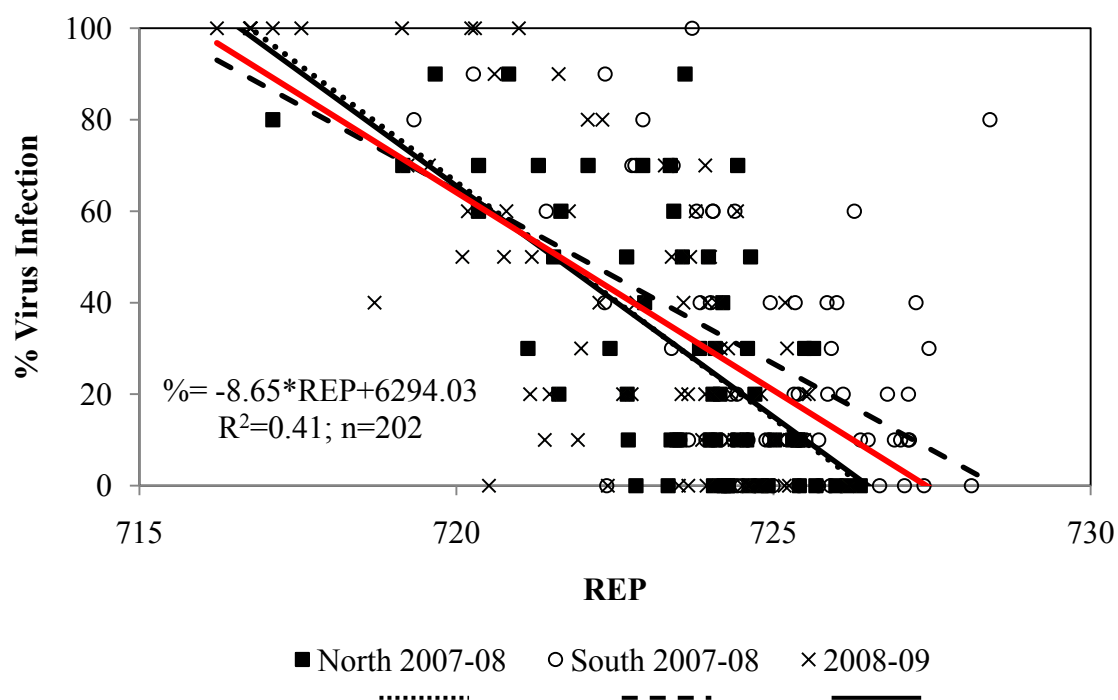




Fig. 1.4. Relationship between percent virus infection and the red edge position (REP). The regression lines of the % infection vs. REP relationship for each data set (black lines) and all data combined (red line) are shown. The regression equation, the coefficient of determination ( $R^2$ ) and the number of samples for all data combined are shown.



## CHAPTER 2

Spatial Pattern of Wheat Curl Mite Movement and Subsequent Virus Spread in Winter

Wheat

## Introduction

The wheat curl mite, *Aceria tosichella* Keifer, is a small eriophyid mite that is widely distributed in North America (Oldfield 1970). The wheat curl mite (WCM) is able to survive on a variety of hosts including many native annual grasses (Connin 1956b), maize (Sill and del Rosario 1959), sorghum (Gibson 1957), barley, and rye (Seifers et al. 1996, Seifers et al. 1997); however, its preferred host is winter wheat. Typically, WCMs develop in protected areas of the plant such as the leaf whorl, axil, or sheath and eventually on green tissue in the head (Peairs 2006).

WCMs feed on cells just below the leaf cuticle (Sabelis and Bruin 1996) and prevent bulliform cells from enlarging; subsequently, the actively growing leaf is prevented from uncurling (Styer and Nault 1996). Mites may cause rolling of the entire leaf, resulting in curled, looped, and trapped leaves (Slykhuis 1955, Somsen and Sill 1970). WCMs cause damage to plants by withdrawal of nutrients, reduction of gas exchange and photosynthesis, death of epidermal cells, and distortion of plant or leaf growth (Sabelis and Bruin 1996). Yield can be reduced by less than 1% to more than 15% by natural WCM infestations (Harvey et al. 2000). Although the WCM has a direct effect on wheat yield, more extensive damage and yield loss is associated with its transmission of wheat streak mosaic virus (WSMV) and two other viruses. High Plains virus (Seifers et al. 1997) and Triticum mosaic virus (Seifers et al. 2008) are also vectored by the WCM; however, WSMV is the predominant WCM-vectored disease in winter wheat in Nebraska (Burrows et al. 2009).

WSMV is potentially the most devastating disease of winter wheat in the central Great Plains and losses can range from negligible to complete crop failures. Resistant wheat varieties (Seifers et al. 2006, Seifers et al. 2007, Martin et al. 2007, Graybosch et al. 2009) and cultural practices (Slykhuis et al. 1957, Thomas et al. 2004, Hesler et al. 2005, Jiang et al. 2005) have been developed to manage the WCM. However, the most important tool available for management of WCMs and subsequent virus infection is the control of pre-harvest volunteer wheat.

In order to survive through the summer after wheat harvest, WCMs must utilize an alternate host (Connin 1956a). In the central Great Plains, volunteer wheat is the most important host utilized by WCMs as a ‘green bridge’ between summer harvest and fall emergence of the new crop (Wegulo et al. 2008). Pre-harvest hail storms cause grain to be shattered on the ground, and this leads to the production of volunteer wheat (Staples and Allington 1956). Volunteer wheat produced in this way germinates and grows quickly, and when maturing wheat plants begin to dry back, WCMs disperse by wind to the new volunteer plants (Staples and Allington 1956, Gibson and Painter 1956).

To efficiently manage the WCM and its vectored viruses, growers must be able to predict the risk of an epidemic occurring in their fields. Wheat curl mites utilize the wind for dispersal (Staples and Allington 1956, Coutts et al. 2008); therefore, the risk of mite movement and virus spread is dependent on the distance of the field from the source population. WCM movement is also dependent on the size of the source population (Thomas and Hein 2003). An increased understanding of mite movement and virus spread will assist in establishing the risk of mite movement into fall-planted wheat.

Growers can use risk zones to more efficiently control volunteer wheat, or if volunteer wheat lies outside the growers control, growers can use virus-resistant varieties or delayed planting to decrease the risk of their fields becoming infected with WCM-vectored viruses. The WCM is small and hard to sample and track; however, WCM-vectored viruses produce significant symptoms that can be visually detected.

Like many plant viruses, WSMV interferes with chloroplast development of systemically infected wheat (White and Brakke 1983, Brakke et al. 1988). Pigments, macromolecules, proteins, and nucleic acids are disrupted in chloroplasts and chlorophyll is reduced in infected leaves (Brakke et al. 1988). Symptoms of WCM-vectored viruses are similar and include yellow to light green streaking, spotting, or mottling (Watkins 2002, de Wolf and Seifers 2008, Wegulo et al. 2008). Symptoms generally appear and become more severe in the spring as temperatures rise in April or May (Staples and Allington 1956). Symptoms of plants that become infected in the fall during early growth stages are more severe and include stunting, rosetting and discoloration.

Remote sensing has been utilized to detect changes in plant response caused by various stresses such as those due to moisture, nutrients, pests, and pathogens (Jones and Schofield 2008). Changes resulting from plant stress affect the amount and direction of radiation reflected and emitted from plants. The red edge position (REP) vegetation index has been used effectively to detect WCM-vectored virus symptoms proximally in this system (Chapter 1). Utilizing remote sensing and the REP index to detect virus symptoms on a larger spatial scale may afford the opportunity to quantify the spatial pattern of WCM movement and subsequent virus spread.

Spatial interpolation has been performed effectively to improve the accuracy of predicting values at un-sampled sites using cokriging (Van der Meer 1998, Estrada-Peña 1999, Tarr et al. 2005, Mutanga and Rugege 2006). Interpolation is based on the assumption that objects that are closer together tend to have similar characteristics. Cokriging is an interpolation method that utilizes two or more spatially interdependent variables: a primary variable of interest that is relatively under sampled, and a secondary variable (or variables) that are often oversampled compared to the variable of interest. In remote sensing studies, the primary variable is generally field observations, while the secondary variable is remote sensing imagery (Van der Meer 1998).

A better understanding of the direction, distance, and spatial pattern of mite movement will enhance our ability to predict the risk of a virus epidemic and allow growers to make better decisions regarding WSMV management. The objective of the current study was to use remote sensing to spatially characterize the spread of WCM-vectored viruses. To meet this objective we needed to determine if the REP index derived from airborne remote sensing data could be used efficiently to estimate biophysical variables associated with WCM-vectored viruses and to determine the spatial pattern of WCM movement and virus spread from variable WCM source population densities using cokriging. The spatial data generated that illustrates mite movement and virus spread in these small scale studies will be used to make predictions of virus spread under field conditions.

## **Materials and Methods**

**Study site.** Simulated volunteer wheat was established by planting two small plots (ca. 10 m x 10 m) in the middle of a fallow wheat field in western Nebraska on 13 July, 2006 (41°14'06"N, 103°00'33"W), 12 July, 2007 (41°14' 16" N, 103° 00' 04" W), and 21 July, 2008 (41°14' 21" N, 102° 59'57" W). Plots were separated by at least 120 meters. Once wheat established, plots were infested with WCMs obtained from other sources of pre-harvest volunteer wheat. To infest plots with WCMs, locations of winter wheat that had suffered pre-harvest hail storms were identified and samples of pre-harvest volunteer wheat were collected from these locations to determine if plants were infested with WCMs. Once a good source population was located, volunteer wheat was collected from that location. The collected wheat infested with WCMs was spread over the growing volunteer source plots. To create a variable infection rate, the amount of biomass used to infest source plots was variable. Two plots were infested by spreading biomass over growing volunteer wheat plots on 10 August, 2006, 14 August, 2007, and 26 August, 2008.

Once volunteer wheat was infested with WCMs, source plots were sampled periodically (ca. 4-5 times) through mid-November (See Table 2.1). Twenty plants were collected from each source plot, and the number of mites per tiller was determined for all tillers of each plant in 2006 and for two tillers per plant in 2007 and 2008. The number of mites per tiller was averaged for each sampling date.

A variety of wheat susceptible to WSMV was planted surrounding the volunteer plots on 20 September, 2006 (cv. Goodstreak; 9.5 ha), 8 September, 2007 (cv. Millenium;

12.7 ha), and 16 September, 2008 (cv. Overland; 8.8 ha). A uniform grid of sampling points consisting of concentric circles was established around each plot. Sampling points were established and flagged at 7.6 m increments in each cardinal direction from the center of each plot and in lines 45° from the four cardinal directions. Eight sampling points resulted in a ring at each 7.6 m increment from the center of the volunteer plot.

In 2006-07, three intermediate sampling rings were added between 7.6 and 15.2 m and one sampling ring was added between 15.2 and 22.9 m. There were five sampling rings between 7.6 and 15.2 m from the center of the north plot and seven sampling rings between 7.6 and 22.9 m from the center of the source plot. In 2007-08 and 2008-09, additional sampling points were added in the spring to fill out gaps in the sampling pattern and increase the likelihood of capturing the extent of variation within plots. In the spring of 2007-08, intermediate sampling rings were added between the sampling rings at 7.6 m and 15.2 m and between the sampling rings at 15.2 m and 22.9 m to more effectively capture the gradient of virus symptoms. In 2007-08, seven rings were located between 7.6 and 38.1 m from the center of the north source plot and eight rings were located between 7.6 and 45.7 m from the center of the south source plot. Due to serious weed infestations present across much of one plot area, only one plot was utilized in 2008-09 and eight sampling rings were located between 7.6 and 61.0 m from the center of the source plot. In the spring, 128 additional sampling points were added to more efficiently describe the gradient of virus symptoms. Additional sampling locations were added where differences in virus symptoms were visible between sampling points. All sampling points were geo-referenced with sub-meter accuracy (Trimble TSCe, Tripod Data Systems Inc.).



A weather station (CR10X datalogger, Campbell Scientific, Inc., Logan, UT) was erected in the middle of one volunteer source plot in September of each year to determine weather conditions during mite movement. The average daily temperature and the average hourly wind direction were calculated between 27 September and 30 November of each year.

**Biophysical measurements.** Ten wheat plants were randomly selected near each flagged sampling location, bagged, and returned to the laboratory in 2006 (north: 5 December, n=54; south: 16 November, n=92), 2007 (north: 15 November, n=76; south 5 December, n=76), and 2008 (8 December, n=76). Three tillers from each plant were viewed under a stereo binocular microscope (magnification ca. 30-40X), and the number of mites per tiller was recorded. Samples were inspected for mites from each sampling ring until mites were no longer detected. The mean number of mites per tiller (MPT) was determined by averaging the number of mites on each of the 30 tillers counted per sampling point. The percentage of tillers infested per sampling point was also determined.

When virus symptoms appeared in the spring, measurements of relative chlorophyll content, leaf area index (LAI), and virus incidence were taken. Relative chlorophyll content measurements were taken on 4 June, and the majority of LAI measurements were taken on 1 and 4 June, 2007 (north, n=43; south, n=67); however, a few LAI measurements were delayed by approximately one week due to unfavorable weather. Relative chlorophyll content readings in both plots and LAI measurements in the south plot were taken between 3 June and 6 June, 2008 (north: n=62; south: n=70).

LAI readings taken in the north 2008 plot were delayed by approximately two weeks due to unfavorable weather conditions. Relative chlorophyll content readings were taken between 18 and 19 June and LAI readings were taken between 8 and 15 June, 2009 (n=216). Relative chlorophyll content and LAI readings were taken from the original sampling locations as well as the 128 additional sampling locations added in the spring to increase the efficiency of describing the gradient of virus spread.

Relative chlorophyll content was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd. Osaka, Japan). Readings were taken from 20 randomly selected flag leaves at approximately one-third the distance from the stem to the end of the flag leaf and averaged to determine the relative chlorophyll content reading at each sampling location. Leaf area index, the ratio of foliage area to the ground, was determined at each sampling location with the LAI-2000 Plant Canopy Analyzer (Li-Cor, Inc., Lincoln, NE). LAI was determined with one above-canopy reading and four below-canopy readings made along a diagonal transect between rows.

After relative chlorophyll content and LAI values were determined, ten tillers were randomly selected at each sampling location, bagged, returned to the laboratory, and frozen until virus incidence could be determined. Samples for virus incidence were collected on 8 June, 2007 (north: n=43; south: n=67), between 4 and 6 June, 2008 (north: n=62; south: n=70), and on 23 June, 2009 (n=76). Virus incidence in each tiller was determined using enzyme-linked immunosorbent assay (ELISA) for WSMV (Seifers et al. 1997, Seifers et al. 2002).

Samples were prepared by extracting approximately 0.2 g frozen plant tissue from the flag leaf with a leaf press in 1.3 mL coating buffer (pH 9.6; 1.59 g  $\text{Na}_2\text{CO}_3$ , 2.93 g  $\text{NaHCO}_3$ , brought to one liter with distilled water). Two plant samples known to be positive for WSMV were used in each plate to verify the effectiveness of the ELISA run. Two control samples from healthy wheat were also used in each plate. For each sample, 200  $\mu\text{L}$  of plant extract from each sample were added to each of two sample wells of an ELISA plate (flat bottom 96-well ELISA plates, Immulon, Dynex Technologies, Chantilly, VA). Plates were incubated at 37°C for one hour then rinsed three times (one minute/rinse) in PBS wash (pH 7.4; phosphate-buffered [PBS] saline and 0.05% Tween 20). Next, 200  $\mu\text{L}$  (diluted 1:4000) antiserum of WSMV were added to each sample well and plates were incubated for one hour at 37°C then rinsed as described above. Following rinses, 250  $\mu\text{L}$  of blocking buffer in 1X PBS (5.0 g nonfat dry milk; 10  $\mu\text{L}$  antifoam A; brought to 100 mL with 1X PBS) were added to each well, incubated for one hour at 37°C and rinsed as described above. Next, 200  $\mu\text{L}$  goat anti-rabbit IgG-alkaline phosphatase label diluted 1:3000 in dilution buffer (pH 7.4; 0.5 ml Tween 20, 20 g PVP, 2.0 g Ovalbumin, 0.2 g sodium azide, brought to one liter with distilled water) were added to each well followed by one hour incubation at 37°C, and rinsed as described above. Finally, 200  $\mu\text{L}$  phosphatase substrate (0.714 mg/ml) were added to each well and incubated at room temperature for one hour.

Absorbance of each ELISA plate was read at 405 nm (MR 4000 Micro ELISA plate reader; Dynatech Laboratories, Chantilly, VA). Plants were considered positive for WSMV when the mean absorbance of the replicate samples was greater than two times the mean absorbance of negative controls in the sample plate.

On 16 July, prior to wheat harvest, 2009, one row by 0.30 m (1ft<sup>2</sup>) samples were taken at each sampling location to determine yield (n=76). Samples were returned to the laboratory and threshed by hand. Samples were cleaned, grain was weighed, and grain weight (g) was converted into kg/ha.

**Hyperspectral Measurements.** Hyperspectral images were collected on 8 June, 2007, 30 May, 2008, and 22 June, 2009. Wheat was in the flowering stage in 2007 and 2008, and the late milk to early dough stage in 2009 when imagery was collected. All images were collected between 0830 and 1200 hours CST. Hyperspectral images were acquired with a plane-mounted AisaEAGLE hyperspectral sensor (Spectral Imaging Ltd., Oulu, Finland) by the Center for Advanced Land Management Information Technologies (CALMIT, University of Nebraska – Lincoln). The AisaEAGLE collects spectral data between 400 and 970 nm. The spectral resolution is 2.9 nm and the slit width is 30 microns. Images were acquired at 1.00 m spatial resolution. Images were corrected for atmospheric effects with Fast Line-of-Sight Atmospheric Analysis of Spectral Hypercubes (FLAASH) and georectified with ground control points.

**Data Analysis.** The values for the biophysical variables in each sampling ring were averaged and plotted against distance to determine the extent of spread and differences in spread between plots. In 2009, the reflectance values for individual pixels in the AisaEAGLE image that had 0% (n=13), 50% (n=6) or 100% (n=7) virus incidence were averaged and plotted against wavelength to determine differences in spectral reflectance between infected and non-infected plants.

The red edge position (REP) index (Guyot and Baret 1988) was calculated for each pixel of each image.

$$REP = 700 + 40 \frac{\rho_{rededge} - \rho_{700nm}}{\rho_{740nm} - \rho_{700nm}}$$

Where:  $\rho_{rededge} = \frac{\rho_{670nm} + \rho_{750nm}}{2}$

Georeferenced sampling locations utilized for biophysical measurements were located on the image and REP values were recorded for each sampling point. Correlation coefficients between biophysical variables and between the biophysical variables and the REP index were calculated for each data set.

Cokriging was utilized in ArcMap 9.2 (ESRI 2006) to develop a prediction map for each data set. A spherical model with a nugget component was used fit to the semivariogram. We hypothesized that WCM movement and symptoms of WSMV were directionally dependent. WCMs utilize wind as a dispersal mechanism, and the prevailing winds in western Nebraska are from the northwest. When anisotropy is applied to models that are directionally dependent, the lines of the model appear spread out, rather than on top of each other. Each model was checked for anisotropy, and the direction of anisotropy associated with each model, to determine the direction that spatial structure was more prevalent.

## Results and Discussion

Weather data were collected to record the conditions during WCM dispersal from source volunteer plots. The mean daily temperature between 27 September and 30 November was 6.3°C in 2006, 7.8°C in 2007, and 7.6°C in 2008. The mean hourly wind direction between 27 September and 30 November was 243° (approximately southwest; Table 2.2; Table 2.2). For wind speeds over 4.5 m/s, the mean hourly wind direction was 273° (west), and at wind speeds over 9 m/s, mean hourly wind direction was 316° (northwest; Table 2.2).

The mean number of mites per tiller (MPT) in source volunteer plots varied between years and between plots (Table. 2.1). The average number of MPT peaked at different sampling dates over the data sets; however the highest peak and the highest average number of MPT over sampling dates after wheat emergence occurred in 2008-09. The 2006-07 north plot and 2007-08 south plot were not significantly different when the mean number of MPT after wheat emergence was averaged, but were significantly lower than the other three plots according to the Student-Newman-Keuls test. The mean number of MPT after wheat emergence in the 2006-07 south plot and the 2007-08 north plot were not significantly different, but were significantly lower than the 2008-09 plot according to the Student-Newman-Keuls test. The mean number of MPT after wheat emergence over all collection dates was highest in the 2008-09 plot followed by the 2006-07 south plot and the 2007-08 north plots, then by the 2006-07 north and 2007-08 south plots.

Spread of WCMs and subsequent virus symptoms was minimal for the 2006-07 plots (north and south). Mean relative chlorophyll content values measured by the SPAD meter and mean LAI values were not significantly different between sampling rings surrounding the source volunteer plot according to the Student-Newman Keuls test. There was a visible pattern of virus symptoms surrounding volunteer source plots in the spring, and virus symptoms were more visible south and east of plots; however, visible symptoms were limited to a few meters around the edges of source volunteer plots. Although the mean number of MPT in source plots after wheat emergence was 43.1 in the 2006-07 north plot and 136.0 in the 2006-07 south plot, and density of WCM source populations and distance from source populations are important to WCM movement, temperature also affects spread of WCMs from source populations. Warm, dry weather during fall months results in increased reproduction and spread of WCMs and buildup of virus in the plant (Wegulo et al. 2008). The mean daily temperature between 27 September and 30 November was 1.5°C lower in 2006 than in 2007 and 1.3°C lower in 2006 than in 2008. Temperature during fall months may have limited WCM movement and subsequent virus spread in 2006-07.

The correlation coefficients were low between the REP index and the biophysical variables in the 2006-07 data sets. A cokriging model could not be fit to any variable in the 2006-07 data sets because the data did not display any spatial pattern. Although virus symptoms spread out in an oval shaped pattern displaced to the southeast, visible symptoms were only noticeable approximately 7-10 m from the center of the source plot. This limited spread that encompassed a limited number of sampling points in our sampling grid resulted in our inability to describe the spatial pattern of spread, low

correlation between biophysical variables and REP, and a lack of spatial correlation from cokriging. Because we could not characterize the spatial pattern of virus spread, the 2006-07 data were not used in further analysis.

The mean percent of tillers infested with WCMs in sampling rings in November and December of 2007 and 2008 and the mean percent of tillers in sampling rings infected with WSMV in 2008 and 2009 were highest near the WCM source plots in each data set and decreased with distance (Appendix B). The 2008-09 plot had a higher mean percentage of tillers in sampling rings infested with WCMs and infected with WSMV than either of the plots in 2007-08, and WCMs and virus moved farther from the source plot in that year. Similar patterns of spread were noted for relative chlorophyll content and LAI.

SPAD readings for healthy plants generally run in the 40's, and SPAD readings below 30 result from plants with severe symptoms. SPAD readings were between 25 and 48.2 in the 2007-08 north plot, 28.2 and 47.9 in the 2007-08 south plot, and 10.6 and 44.7 in the 2008-09 plot. LAI values were between 0.97 and 4.07 in the 2007-08 north plot, 1.7 and 3.9 in the 2007-08 south plot, and 0.72 and 4.16 in the 2008-09 plot. Mean relative chlorophyll content and LAI readings in each sampling ring were lower near the source plots and increased with distance (Appendix B). Mean relative chlorophyll content in each sampling ring was lower in the 2008-09 plot (range: 15.9-39.1) compared to the 2007-08 plots (range: 33.9-42.2). Mean LAI readings in each sampling ring were lower in the 2008-09 (range: 1.2-2.7) and 2007-08 north plot (range: 1.4-2.3) compared to the 2007-08 south plot (range: 2.6-2.8). Variation of mean LAI readings between



sampling rings was limited in the 2007-08 south plot. Mean yield in each sampling ring increased with distance from the source plot in the 2008-09 plot, the only year that yield samples were taken (Appendix B).

In each year, a significant pattern of virus symptoms was visible surrounding plots. Chlorosis was noticeable close to the source plots in 2007-08. Although chlorosis was noticeable in tillers located further from the source plots, the majority of yellowing was located within 15 m of the source plot. Some plants were observed to be stunted close to source plots; however, stunting was not visibly noticeable beyond approximately 12-15 m from the source plot. Chlorosis and stunting formed an oval pattern surrounding the source plots which was displaced to the southeast. Chlorosis was more visible in the north plot when compared to the south plot in 2007-08 (Appendix C).

Significant virus symptoms were observed in the 2008-09 plot (Appendix C). Plants closest to the source plots were severely infected and yellowing remained visible approximately 15-23 m from the source plots. Chlorosis continued to be visible approximately 30-38 m from the source plot. Stunting was observed within the first 15-23 m. Symptoms were distributed in an oval pattern that was displaced to the southeast.

Reflectance spectra of AisaEAGLE raw bands at sampling locations with 100% and 50% WSMV exhibited differences in spectral reflectance that can be discriminated from uninfected sampling locations. The average spectral profiles of wheat samples that had 100%, 50% or 0% virus incidence in 2008-09 differed at certain wavelengths (Fig. 2.1). The highest degree of separation between uninfected and 100% infected samples was located, by visual observation, in the red edge of reflectance, between 670 and 780

nm. This was followed by selected ranges in the near infrared (NIR; 760-920 nm), red (650 nm) and green (550 nm) regions.

The majority of biophysical variables measured were significantly correlated to each other (Table 2.3). Leaf area index in the 2007-08 south plot did not have a significant relationship with any of the biophysical variables tested. As MPT, percent of tillers infested with WCMs, and percent virus infection increased, relative chlorophyll content, LAI, and yield decreased. MPT, percent tillers infested with WCMs, and percent virus infection decreased with distance while relative chlorophyll content and LAI increased with distance from the WCM source plot.

The REP index was significantly correlated with all biophysical variables (Table 2.3). The wavelength of the REP decreased as MPT, percent tillers infested with WCMs, and percent virus infection increased, and REP increased as relative chlorophyll content, LAI, and yield increased. The REP moved towards longer wavelengths as distance from the WCM source plot increased.

Cokriging was utilized to determine the fit of the model between each of the biophysical variables (the primary variable) and the REP index (the secondary variable) for each data set. Cokriging calculates predictions for the under sampled, or primary variable with the help of the oversampled variable, or the secondary variable (Mutanga and Rugege 2006). It utilizes a linear model of coregionalization that makes use of both the spatial autocorrelation in the primary variable and the cross-correlation between the primary and secondary variable (Hudak et al. 2002).

In order to fit a model with cokriging, data must be spatially correlated.

Cokriging uses the semivariance to express the degree of relationship between points on a surface. The semivariance is the average of the squared differences in values between all possible points spaced a constant distance apart. When data are spatially correlated, the semivariance increases as points are compared to increasingly distant locations; therefore, locations that are closer together have more similar values and as pairs of locations become further apart, they are less similar and have a higher squared distance. If there is no spatial correlation, the semivariogram is flat.

REP was spatially correlated with percent virus infection and relative chlorophyll content in all three plots. REP was spatially correlated with LAI and percent tillers infested with WCMs in the 2007-08 north and 2008-09 plots. REP was spatially correlated with yield in 2008-09; the only year yield was measured. Because these variables were spatially correlated, it was possible to fit a model between REP and these variables with cokriging. The mean number of MPT was not spatially correlated with REP in any plot and a model could not be fit between MPT and REP.

Cross-validation was utilized to determine the accuracy of each model. In cross-validation, each point in the sampling scheme is removed individually and its value is predicted by cokriging the remaining data (Tarr et al. 2005). For all points, cross-validation compares the measured and predicted values. The coefficients of determination ( $R^2$ ) were determined by regressing the measured values and the values predicted by cokriging for each model in each data set (Table 2.4).

Because I was interested in determining the extent of WCM movement and subsequent WSMV spread from the source plots, percent virus infection was determined to be the most useful primary variable to utilize in the cokriging model. Both percent virus infection and relative chlorophyll content had a spatial relationship with REP in all three plots; however, the coefficients of determination were higher for percent virus infection than for relative chlorophyll content for the 2007-08 plots. The  $R^2$  was higher for relative chlorophyll content ( $R^2=0.82$ ;  $n=214$ ;  $P<0.0001$ ) than percent virus infection ( $R^2=0.74$ ;  $n=74$ ;  $P<0.0001$ ) in the 2008-09 plot. By using percent virus infection as the primary variable in cokriging, a prediction map can be created that displays the spatial pattern associated with specific classes of virus infection. Chlorosis, however, is also highly associated with WCM-vectored viruses (Brakke et al. 1988, Wegulo et al. 2008) and the SPAD meter is an efficient tool for measuring relative chlorophyll content (Kariya et al. 1982, Yadava 1986). The geographic information system utilized to perform cokriging allows up to three secondary variables to be utilized. Therefore, both REP and relative chlorophyll content can be used in cokriging to predict percent virus infection.

Cokriging was used to determine if  $R^2$  could be increased by utilizing percent virus infection as the primary variable and both the REP index and chlorophyll measured with the SPAD meter as secondary variables. The addition of relative chlorophyll content as a secondary variable did not greatly affect cokriging in the 2007-08 data (north:  $R^2=0.56$ ; south:  $R^2=0.43$ ) because relative chlorophyll content was not oversampled compared to percent virus infection that year; however,  $R^2$  was increased

from 0.74 to 0.78 and the RMSE was reduced by 7.8% (from 17.28 to 15.93) by adding relative chlorophyll content as a secondary variable in the 2008-09 data set.

The sampling scheme utilized to measure relative chlorophyll content was increased dramatically in the 2008-09 plot to more efficiently capture the spatial spread of symptoms associated with WCM-vectored viruses. The additional 141 sampling locations where relative chlorophyll content was measured may have effectively resulted in an increased  $R^2$  for the model between percent virus infection and REP index. In 2007-08, the same number of samples were collected for percent virus infection and relative chlorophyll content. Because the goal was to remain consistent when developing models across data sets, cokriging models were developed for each data set by utilizing percent virus infection as the primary variable, and the REP index and relative chlorophyll content as the secondary variables.

In cokriging, models are either isotropic or anisotropic. Isotropic models have uniformity in all directions while anisotropic models are directionally dependent. Anisotropy characterizes a variable that does not have the same properties in all directions. I hypothesized there would be a spatial pattern that was displaced in the southeast direction because WCMs utilize wind for dispersal and the prevailing winds in western Nebraska are from the northwest. When anisotropy was added to the models, the direction of anisotropy was similar for all three data sets; anisotropy was  $333.7^\circ$  for the 2007-08 north data,  $334.2^\circ$  for the 2007-08 south data, and  $319.2^\circ$  for the 2008-09 data. The direction of anisotropy in all three plots indicates spatial structure is more prevalent in that direction.

The predicted variables determined by cokriging were classified into four groups (0-25%, 25-50%, 50-75%, and 75-100% virus incidence). The results of cokriging are displayed for each data set (Fig. 2.2, 2.3, 2.4). In each map, cokriging predicted the highest percent of virus infection to be closest to the plots, and virus incidence decreased with distance from the source plot. The maps display an oval shaped pattern of virus spread which is displaced to the southeast in each data set and more spread in the northwest-southeast direction. These observations are consistent with the degree of anisotropy in the associated models.

The degree of anisotropy was approximately northwest. Anisotropy was compared to mean hourly wind direction between 27 September and 30 November of each year. The mean hourly wind direction was approximately west to southwest in both years (Table 2.2). This average hourly wind direction would be consistent with predominant winds from the northwest and southeast, and indeed, the predominant winds in the region are from these two directions. The number of hours when wind speed came from the north, northwest, and west was generally higher than the number of hours when wind originated from other directions (Appendix D). The average wind direction was approximately west when wind speed was greater than 4.5 m/s, and approximately northwest when wind speeds were over 9 m/s (Table. 2.2). Wind speeds above 9 m/s are consistent with the degree of anisotropy associated with the cokriging models.

Although WCMs can disperse at any wind speed, mites may disperse more frequently, or disperse farther, when wind speeds are higher. For all hours in 2007-08, and all but one hour in 2008-09 when wind speeds were above 9 m/s, wind direction was

between north and west (270°-360°). Both visible symptoms in the field and predictions by cokriging display virus symptoms to be more pronounced southeast from the source plots. A pattern of virus spread that is more prevalent in the direction of the highest wind speeds may indicate that WCMs disperse more readily when wind speeds are higher. This suggests that WCMs can sense changes in weather conditions associated with higher wind speeds that increase dispersion.

Barometric pressure changes are associated with the passing of a cold front. Barometric pressure decreases as cold fronts approach and increases as the front passes. This often results in strong northwest winds associated with the high pressure area. Changes in barometric pressure have been found to affect dispersal of the two spotted spider mite, *Tetranychus urticae* Koch (Li and Margolies 1994). Early dispersal was associated with rising barometric pressure, and Li and Margolies (1994) hypothesized that *T. urticae* initiates aerial dispersal from deteriorating habitats in response to cues signaling favorable weather conditions. WCMs may also initiate dispersal behavior in response to changes in weather such as this.

To determine the approximate distance of virus spread predicted by cokriging, the distance between the centers of the source volunteer plots to the edge of each virus infection class was measured with the measuring tool in a GIS. Measurements were taken in each of the four cardinal and four ordinal directions (Table 2.5). Measurements were taken conservatively and the closest location to the source plot where class changed in each direction was determined to be the edge of the class. For all three plots, the distance each class extended from the edge of the source plot was highest in the southeast

direction. Overall, the highest class of virus (75-100%) that spread farther from the edge of the source plot was in the 2008-09 plot (23.92 m), followed by the 2007-08 north plot (10.67 m) and the 2007-08 south plot (8.74 m).

The source plot was excluded from the cokriging map, and the area each class encompassed was calculated for each plot (Table 2.6). The area of spread for 75-100% and 50-75% virus infection was higher in the 2008-09 plot, followed by the 2007-08 north and 2007-08 south plots. As mentioned previously, WCM movement and subsequent WSMV spread are dependent both on the distance from the source plot, and the density of the source population. The mean number of MPT in source plots in the fall of each year was highest for the 2008-09 plot, followed by the 2007-08 north and 2007-08 south plots. These results indicate the source populations with the highest average number of WCMs per tiller resulted in the largest spread of virus in the spring.

The results of this study can be utilized to extrapolate the potential spatial spread of WCMs and subsequent virus infection to a field-size scale. For example, if the 100 m<sup>2</sup> source plot utilized in this study were increased to 32.4 ha (80 Acres), and the source population of WCMs and weather conditions were similar to those in 2008-09, data in Table 2.4 can be utilized to predict the spatial spread of virus infection. Assuming WCM movement and subsequent virus spread is linear, under these conditions, in the southeast direction, 75-100% of tillers have the potential to be infected 1.4 km from the center of the source field, 50-75% of tillers have the potential to be infected 2.2 km from the center of the source field, and 25-50% of tillers have the potential to be infected 3.3 km from the center of the source field. The validity of the assumption that a linear increase with



increasing scale for this relationship occurs is very difficult to establish. However, mite movement is extremely hard to measure, and this assumption allows an estimate of movement and virus risk to be made. Future efforts that use remote sensing to track virus presence may be used to validate these virus spread estimates.

## Conclusions

The REP index derived from aerial remote sensing can efficiently estimate biophysical variables associated with WCM-vectored viruses, and it was effective in producing prediction maps of the spatial pattern of virus spread from WCM source populations. WCMs disperse by wind, and the prevailing wind pattern in western Nebraska where this study was located is from the northwest. Prediction maps developed with cokriging displayed a distinct oval pattern of virus symptoms outside the source plot that was displaced to the southeast, consistent with weather patterns in western Nebraska and with observations of virus spread in the field.

The density of WCMs in the source plot was significantly different between plots, and prediction maps also varied in the extent of virus spread in the cokriging prediction maps. Higher rates of virus infection encompassed a larger area for plots that had a higher number of mites in the source volunteer plot. The spread of virus quantified by cokriging was lower in plots that had a lower number of mites in the source volunteer plot. This technique will be useful for predicting the risk of epidemics occurring near source populations of the virus-vector and the potential distance and spatial structure of virus spread from a source population.

Risk parameters can be used by wheat growers to more effectively manage WCMs. Growers can use this information to evaluate the sphere of influence (virus risk) of volunteer wheat fields and make better decisions to prioritize control of pre-harvest volunteer wheat in areas of high risk. If pre-harvest volunteer wheat is outside of the

grower's control, growers can utilize later planting dates or virus-resistant varieties to help manage their risk.

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**Tables**



Table 2.1. Fall populations of wheat curl mites (mites per tiller) in source volunteer plots.

Mean $\pm$ SE					
	2006-07 North	2006-07 South	2007-08 North	2007-08 South	2008-09
<b>Sample Dates after Plot Infestation</b>					
Date 1	73.06 $\pm$ 10.47 (24 Aug.)	19.84 $\pm$ 3.11 (24 Aug.)	10.62 $\pm$ 2.76 (16 Aug.)	4.40 $\pm$ 1.39 (16 Aug.)	--
Date 2	--	--	22.45 $\pm$ 3.68 (30 Aug.)	10.75 $\pm$ 2.13 (30 Aug.)	26.33 $\pm$ 5.73 (3 Sept.)
Date 3	24.37 $\pm$ 2.87 (8 Sept.)	--	82.63 $\pm$ 8.78 (14 Sept.)	74.25 $\pm$ 12.46 (14 Sept.)	76.15 $\pm$ 11.53 (16 Sept.)
<b>Sample Dates after Wheat Emergence</b>					
Date 4	23.59 $\pm$ 2.15 (27 Sept.)	48.12 $\pm$ 5.95 (27 Sept.)	92.08 $\pm$ 20.60 (26 Sept.)	30.03 $\pm$ 6.80 (26 Sept.)	148.95 $\pm$ 18.40 (30 Sept.)
Date 5	59.16 $\pm$ 10.30 (12 Oct.)	221.92 $\pm$ 27.80 (12 Oct.)	105.43 $\pm$ 21.28 (11 Oct.)	25.33 $\pm$ 4.34 (11 Oct.)	315.43 $\pm$ 31.76 (17 Oct.)
<b>Mean Mites per Tiller after Wheat Emergence</b>					
	43.12 $\pm$ 10.46a	136.04 $\pm$ 10.64b	98.75 $\pm$ 19.07b	27.68 $\pm$ 19.07a	232.19 $\pm$ 19.07c

<sup>a</sup>Means within a row followed by the same letter are not significantly different ( $P > 0.05$ ) according to the Student–Newman-Keuls test.

Table 2.2. Average hourly wind direction at variable wind speeds.

	Hourly Wind Direction		
	Wind speed > 0 m/s	Wind speed $\geq$ 4.5 m/s	Wind speed $\geq$ 9 m/s
2006-07	231.12° (n=1546)	261.37° (n=472)	312.95° (n=29)
2007-08	260.62° (n=1540)	290.02° (n=520)	323.52° (n=67)
2008-09	237.67° (n=1463)	270.13° (n=490)	311.98° (n=99)



(Table 2.3 Continued)

	LAI	% Virus Infection	% Tillers Infested	MPT	Distance	Yield	REP
<b>2008-09</b>							
Chlorophyll	0.66 <sup>*</sup>	-0.82 <sup>*</sup>	-0.84 <sup>*</sup>	-0.64 <sup>*</sup>	0.82 <sup>*</sup>	0.77 <sup>*</sup>	0.79 <sup>*</sup>
LAI	--	-0.71 <sup>*</sup>	-0.70 <sup>*</sup>	-0.51 <sup>*</sup>	0.69 <sup>*</sup>	0.75 <sup>*</sup>	0.81 <sup>*</sup>
% Virus Infection	--	--	0.82 <sup>*</sup>	0.60 <sup>*</sup>	-0.77 <sup>*</sup>	-0.68 <sup>*</sup>	-0.72 <sup>*</sup>
% Tillers Infested	--	--	--	0.83 <sup>*</sup>	-0.71 <sup>*</sup>	-0.74 <sup>*</sup>	-0.72 <sup>*</sup>
MPT	--	--	--	--	-0.50 <sup>*</sup>	-0.61 <sup>*</sup>	-0.49 <sup>*</sup>
Distance	--	--	--	--	--	0.65 <sup>*</sup>	0.73 <sup>*</sup>
Yield	--	--	--	--	--	--	0.81 <sup>*</sup>

<sup>\*</sup>Significant at  $p < 0.05$ .

<sup>a</sup>Leaf area index (LAI).

<sup>b</sup>Mites per tiller (MPT).

Table 2.4. Coefficients of determination ( $R^2$ ) for the cross-validation between each biophysical variable and the red edge position (REP) vegetation index <sup>a</sup>. It was not possible to model mites per tiller (MPT) with cokriging.

Biophysical Variable	2007-08 North	2007-08 South	2008-09
% Virus Infection	0.56 (n=61)	0.42 (n=70)	0.74 (n=74)
Chlorophyll	0.37 (n=61)	0.30 (n=70)	0.82 (n=215)
Leaf Area Index	0.66 (n=62)	-- <sup>b</sup>	0.76 (n=211)
% Tillers Infested	0.22 (n=43)	-- <sup>b</sup>	0.71 (n=76)
Yield	-- <sup>c</sup>	-- <sup>c</sup>	0.59 (n=64)

<sup>a</sup> All values significant at  $p < 0.001$ .

<sup>b</sup> Variable could not be modeled with cokriging.

<sup>c</sup> No yield data collected.

Table 2.5. Approximate distance (m) of each class of virus spread from the center of the source plot in each cardinal and ordinal direction. Distances were calculated from the center of the source plot to the closest edge of each class of infection rates with the measuring tool in a GIS system.

Extent of Virus Spread (m) by Direction								
	N	NE	E	SE	S	SW	W	NW
<b>2007-08</b>								
<b>North</b>								
75-100%	7.58	6.22	8.78	10.67	1.41	1.18	2.68	2.56
50-75%	14.95	10.20	11.31	21.18	6.30	4.75	4.65	6.86
25-50%	23.66	14.35	16.00	30.74	14.63	8.17	11.00	14.76
<b>2007-08</b>								
<b>South</b>								
75-100%	5.16	5.91	4.24	8.74	7.04	5.67	7.50	7.05
50-75%	16.84	14.14	9.37	25.01	14.30	7.05	9.60	14.77
25-50%	22.60	18.08	18.02	28.70	23.49	10.70	12.73	20.51
<b>2008-09</b>								
75-100%	12.76	12.07	17.27	23.92	15.25	5.58	7.36	16.60
50-75%	25.72	13.53	25.79	39.50	32.36	8.46	15.22	26.62
25-50%	49.43	31.99	31.66	57.57	45.67	14.85	30.97	32.25

Table 2.6. Area outside the wheat curl mite source plot predicted by cokriging to be associated with each percent virus infection rate.

	Percent Virus Infection		
	25-50%	50-75%	75-100%
2007-08 North	0.10 ha (0.24 ac)	0.04 ha (0.11 ac)	0.01 ha (0.02 ac)
2007-08 South	0.17 ha (0.41 ac)	0.03 (0.08 ac)	0.004 ha (0.01 ac)
2008-09	0.30 ha (0.75 ac)	0.10 ha (0.24 ac)	0.07 ha (0.17 ac)

## Figures



Fig. 2.1. Reflectance spectra of samples containing various wheat streak mosaic virus (WSMV) levels: (a) mean reflectance for samples with 100%, 50% , and 0% virus infection and (b) difference of means between samples with 100% WSMV and without WSMV.

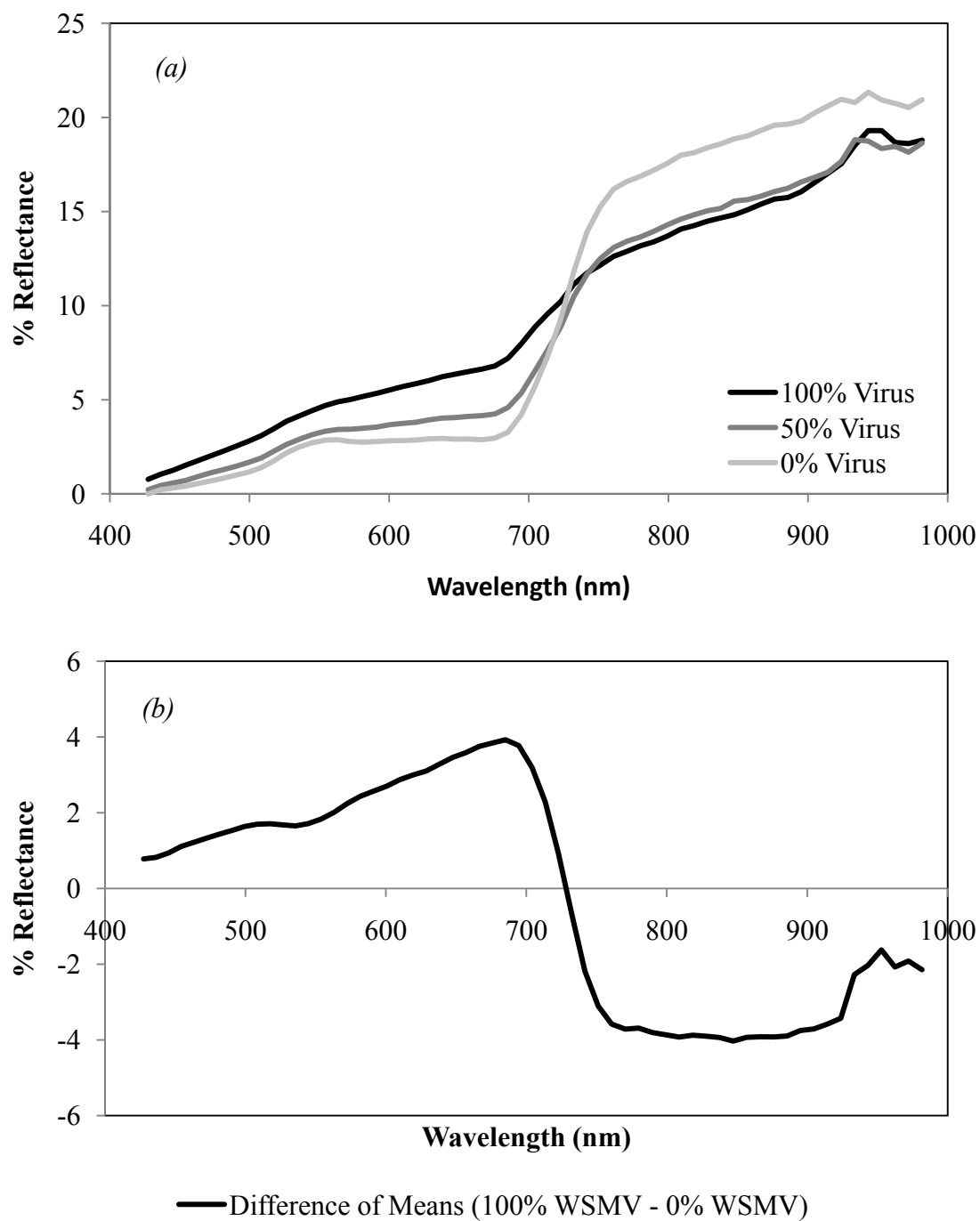


Fig. 2.2. Percent virus infection surrounding the 2007-08 north wheat curl mite source plot predicted by cokriging. The primary variable was percent virus infection and secondary variables were red edge position (REP) and relative chlorophyll content.

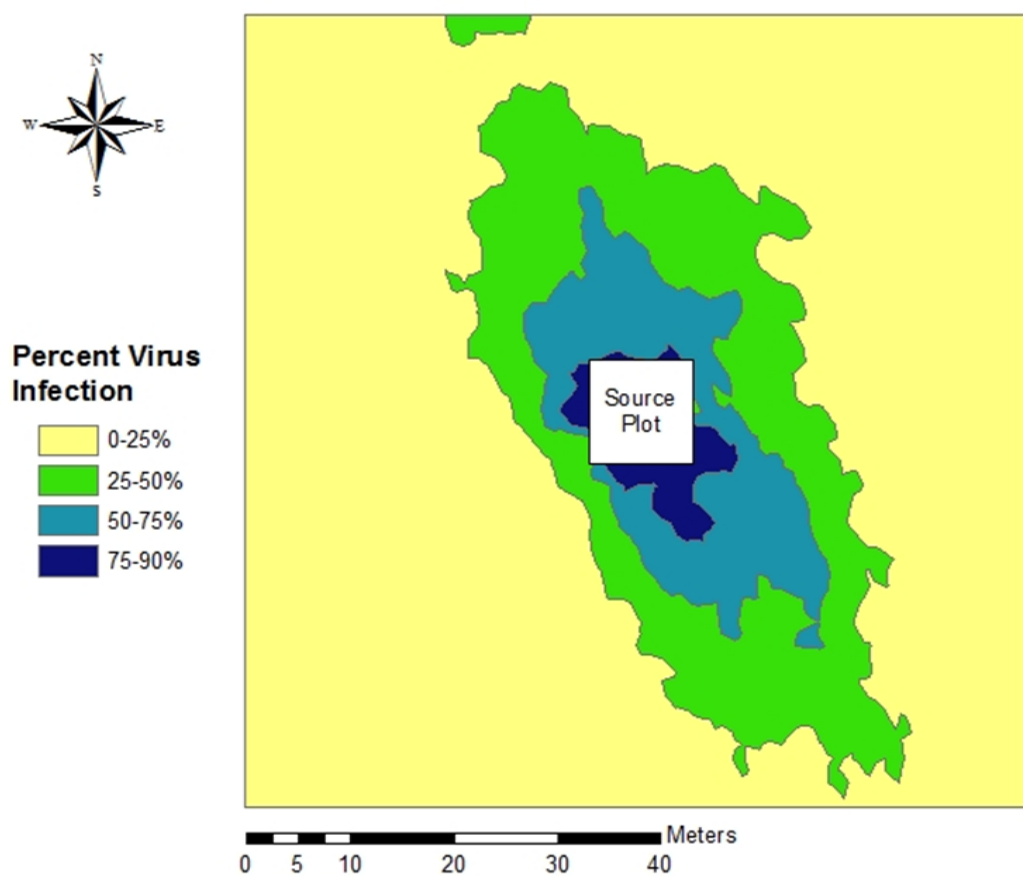


Fig. 2.3. Percent virus infection surrounding the 2007-08 south wheat curl mite source plot predicted by cokriging. The primary variable was percent virus infection and secondary variables were red edge position (REP) and relative chlorophyll content.

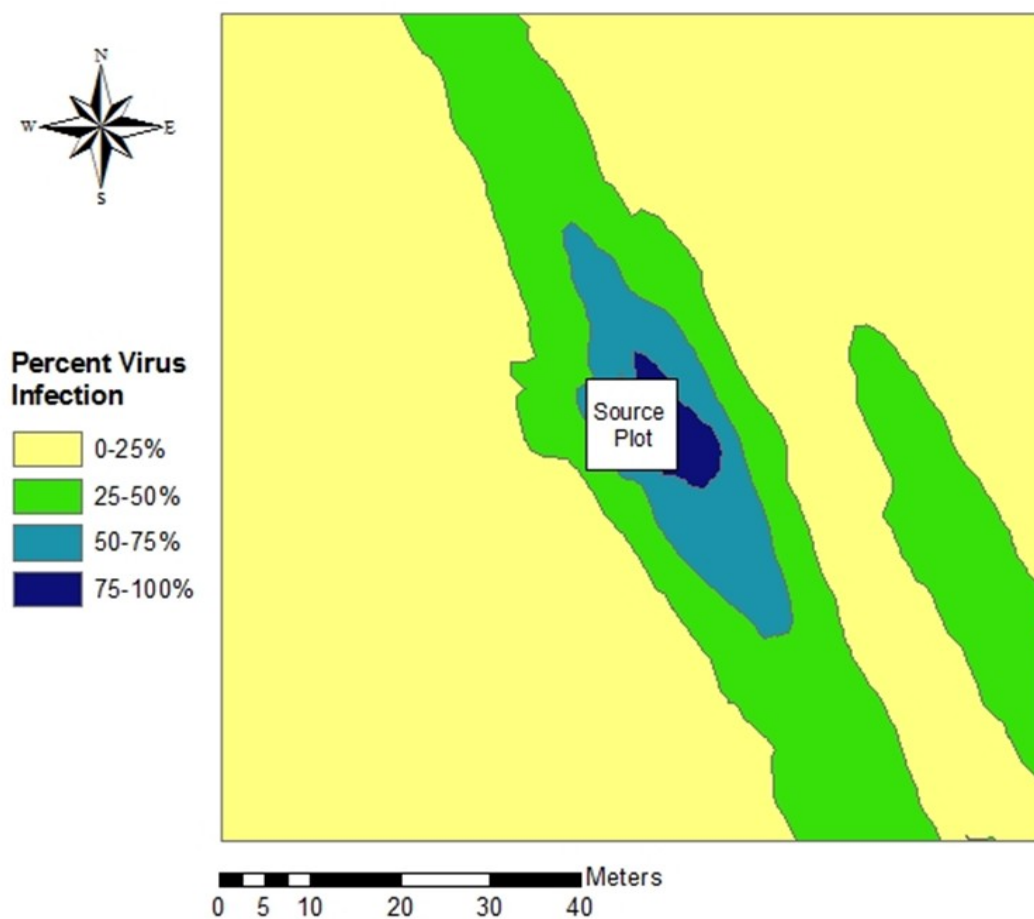
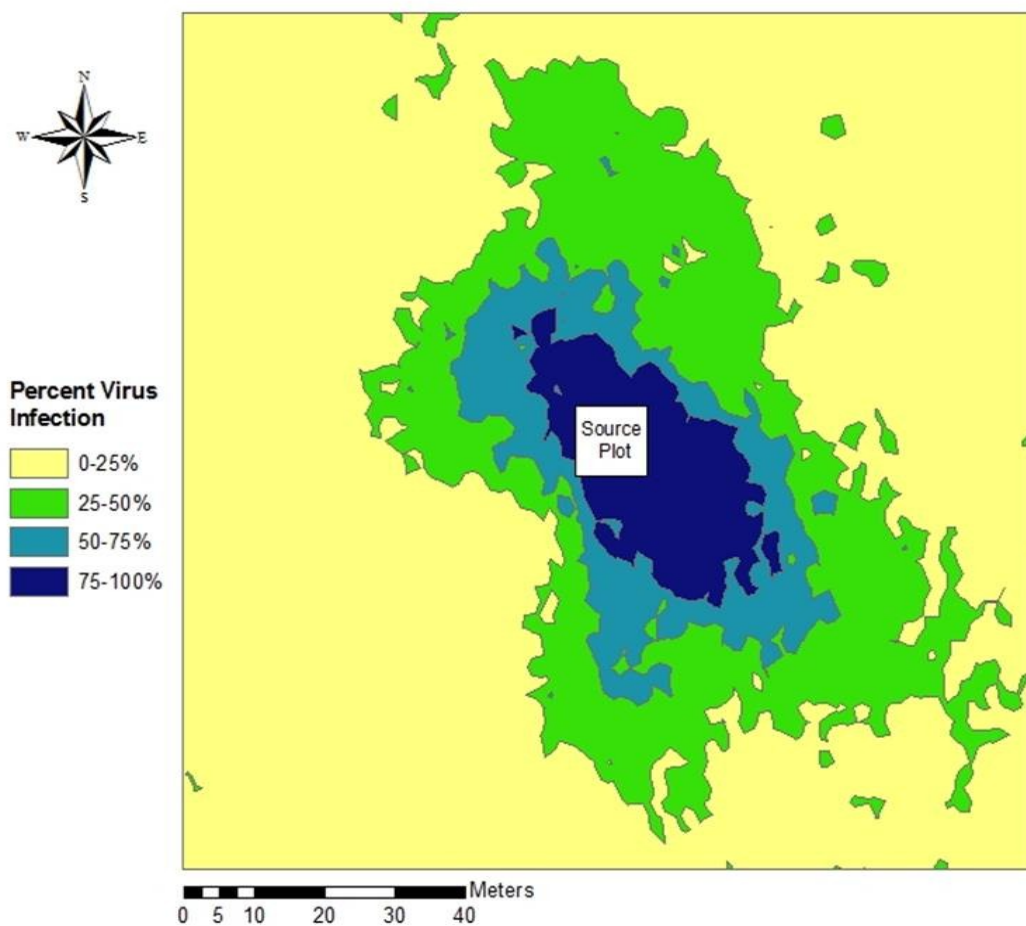


Fig. 2.4. Percent virus infection surrounding the 2008-09 wheat curl mite source plot predicted by cokriging. The primary variable was percent virus infection and secondary variables were red edge position (REP) and relative chlorophyll content.



## CHAPTER 3

## Thrips as Predators of Wheat Curl Mites in Winter Wheat Cropping Systems

## Introduction

The wheat curl mite, *Aceria tosichella* Keifer, is a microscopic eriophyid mite widely distributed in North America (Oldfield 1970). Eggs, immature stages, and adult wheat curl mites (WCM) are found on winter wheat and other nearby annual grasses (Peairs 2006). Typically, WCMs develop in protected areas of the plant such as the leaf whorl, axil or sheath, and later on green tissues in the head (Peairs 2006). WCMs penetrate the cuticle of leaves with their stylets and feed on cells just below the leaf cuticle (Sabelis and Bruin 1996). When infestations of WCMs reach high numbers, yield may be reduced. At levels of 450 WCMs per wheat spike, yield may be reduced from less than 1% to more than 15% (Harvey et al. 2000).

The greatest impact of WCMs results from its transmission of viruses. The WCM vectors three viruses: wheat streak mosaic virus (WSMV) (Slykhuis 1953a, Slykhuis 1953b, Slykhuis 1955), High Plains virus (HPV) (Seifers et al. 1997), and Triticum mosaic virus (TriMV) (Seifers et al. 2008). Symptoms of these viruses include chlorosis and stunting (Watkins 2002, de Wolf and Seifers 2008, Wegulo et al. 2008) and outbreaks of WSMV have caused extensive monetary loss (Sill and Agusiobo 1955, Hershman 2000, Appel et al. 2006).

Resistant wheat varieties (Seifers et al. 2006, Seifers et al. 2007, Martin et al. 2007, Graybosch et al. 2009) and cultural practices (Slykhuis et al. 1957, Thomas et al. 2004, Hesler et al. 2005, Jiang et al. 2005) have been developed to manage the WCM. Currently, there are no reports in the literature concerning natural enemies of the WCM; however, mites are common prey for thrips throughout temperate and tropical regions

(Lewis 1973, Chazeau 1985), and some thrips species traditionally thought of as pest species are reported to also be predaceous (Martin 2000, Sutherland 2006). Obligate predation occurs in some thrips genera and facultative predation is widespread (Mound 2005).

Most tropical thrips in the family Aeolothripidae appear to be obligate predators; however, the majority of related species in the genus *Aeolothrips* in temperate regions are facultative predators in flowers (Hoddle et al. 2004). *Aeolothrips fasciatus* is known to feed on the oat thrips, onion thrips, gladiolus thrips and bean thrips (Lewis 1973) as well as insects in the families Curculionidae, Aphidae, Chloropidae, Cicadellidae, Pteromalidae, Jassidae and Eulophidae (Thompson and Simmonds 1965).

*Frankliniella fusca* (Hinds), *Frankliniella occidentalis* (Pergande), *Thrips tabaci* Lindeman and *Frankliniella schultzei* Trybom are essentially phytophagous, but they can be facultative spider mite predators (Thompson and Simmonds 1965, Trichilo and Leigh 1986, Wilson et al. 1996, Milne and Walter 1997, Milne and Walter 1998). *F. fusca* has been reported to be predaceous on *Tetranychus telarius* (L.) in the United States (Thompson and Simmonds 1965). *F. occidentalis* preferentially feeds on the eggs of *Tetranychus pacificus* McGregor rather than on leaf tissue when eggs are present on cotton, and when plant tissue is previously exposed to spider mite feeding, this effect is enhanced (Agrawal et al. 1999). *T. tabaci* and *F. schultzei* are reported to feed on eggs of *T. urticae* on cotton as a supplementary food (Milne and Walter 1997, 1998). *T. tabaci* and *F. schultzei* that supplement their diet with mite eggs have reduced developmental time from egg to adult and increased fecundity when compared to individuals that feed on

host-plants alone (Milne and Walter 1997, 1998). *F. occidentalis*, *T. tabaci* and *F. schultzei*, however, do not appear to be specifically adapted for predation. Predation may be more influenced by the coincidental presence of the thrips and spider mites on the host than the thrips actively seeking out mite eggs (Trichilo and Leigh 1986, Milne and Walter 1998).

Thrips have also been reported to be predaceous on other thrips and insects. *Franklinothrips vespiformis* (Crawford) is reported to feed on mites, leafhoppers and white fly in citrus and avocado groves in central and South America (Moulton 1932), and occasionally attacks *Dinurothrips hookeri* Hood on ornamental flowers and *Caliothrips insularis* on Sudan grass (Callan 1943). *F. vespiformis* and *Frankliniella tenuicornis* (Uzel) are reported to be predators of cacao and glasshouse thrips (Callan 1943). *Anaphothrips obscurus* (Muller), traditionally found on grasses and cereals (Mound and Kibby 1998), has been reported to feed on the thrips species *Taeniothrips inconsequens* (Uzel) (Thompson and Simmonds 1964).

Thrips are also reported to feed on eriophyid mites. Two species of thrips, *Haplothrips subtilissimus* Haliday and *Xylaplothrips fuliginosus* (Schille) are reported to suppress populations of free-living gall mite species of the Eriophyoidea in Germany (Schliesske 1992). *Leptothrips mali* (Fitch), a predaceous thrips, is known to feed on the tomato russet mite, *Vasates lycopersici* (Masee) (Bailey and Keifer 1943, Anderson 1954). *L. mali* is one of the most common and widely distributed predaceous thrips in North America and has been collected in the majority of continental states (Bailey 1940). The presence of this species often indicates the presence of eriophyids that are difficult to



see, and there is positive evidence of *L. mali* feeding on *Calepitrimerus baileyi* Keifer and *Eriophyes vitis* (Landois), both eriophyid species (Bailey 1940).

Observations in the field and greenhouse suggest a correlation between thrips and WCM populations in western Nebraska (G.L. Hein, personal communication). Mite populations in greenhouse colonies tend to drop rapidly when wheat becomes infested with thrips. In addition, when thrips populations in the field are high, mite density tends to be lower. The objective of this study was to evaluate the potential of thrips as predators of WCMs. Specific objectives were to (1) determine the diversity of thrips present in winter wheat fields in western Nebraska, to (2) determine the impact of thrips on WCM populations in the greenhouse and in the field, and to (3) conduct observational studies to verify WCM predation by thrips.

## Materials and Methods

**Thrips in winter wheat.** Winter wheat fields with different cropping rotations were sampled over two years to determine the density and species of thrips in winter wheat. Western Nebraska winter wheat fields in three counties in 2007 and in two counties in 2008 were sampled for thrips. In 2007, three fields were sampled in Banner County (15 and 27 June), three fields were sampled in Cheyenne County (5 and 26 June), and two fields were sampled in Scotts Bluff County (20 June). In each field, 25 wheat tillers were randomly selected at each of ten random locations.

In 2008, three fields were sampled in Banner County (23 May, 10 and 23 June, and 7 July), and three fields were sampled in Cheyenne County (21 May, 9 and 24 June, and 8 July). In order to increase the sample size and include more thrips, in each field, one row by 0.30 m (1 ft<sup>2</sup>) samples of wheat were taken from 10 randomly selected locations.

Each sample was placed in a plastic bag and sealed. All samples were returned to the laboratory, and immediately placed in Berlese funnels for extraction. After 48 hours, samples were removed from the funnels and the number of tillers per sample was counted.

If the wheat canopy was dry during sampling in 2008, sweep-net samples were taken in four randomly selected locations in each field. Sweep net samples consisted of 25 arcs (each 180°) taken perpendicular to rows, using a 37.5 cm diameter net. Samples were frozen and later inspected for the presence of thrips.

Adults and larvae were counted under a microscope and stored in 70% ethanol.

The mean number of adult, larval, and total thrips per 25 tillers and 25 sweeps in each field was determined with PROC MEANS in SAS (SAS Institute 2002, 2003). All adults in each sample in 2007 and approximately half of adults in each sample in 2008 were identified by Linda A. Mahaffey, Colorado State University, by using the keys and characteristics found in Stannard 1968, Mound and Kibby 1988, and Hoodle et al. 2008.

**Effect of thrips on WCM populations in the greenhouse.** Four greenhouse experiments were conducted to determine the impact of thrips on WCM populations. All experiments began in early- to late-June and were completed by mid-July to early-August. Winter wheat was planted in 24 conetainers approximately 21 cm in length with a 4 cm diameter opening. Seven to eight wheat seeds (var. Alliance) were planted in each conetainer. Each conetainer was fitted with a tubular, clear, plastic cage approximately 31 cm in height with a 4.1 cm diameter opening. Each cage had three holes approximately 5 cm in diameter that were covered with Nitex® screen (225 x 326 mesh; Sefar America, Inc. Depew, NY) impenetrable by WCMs. After planting, conetainers were placed in the greenhouse (temperature 24° to 38° C). Artificial lights were on for 16 h per day during these experiments. After approximately one week, plants were thinned to one tiller per cone in experiments 1 and 2, and two tillers per cone in experiments 3 and 4.

There were two treatments: half of the wheat plants were infested with mites and thrips, while the remaining 12 plants were only infested with mites. The experimental design was completely randomized with four replications and three sampling dates.

Each conetainer was infested with 10 WCMs in experiments 1-3 and 5 WCMs in experiment 4. Mites were obtained from greenhouse colonies. Wheat curl mites were transferred individually to a small, moistened triangle of black filter paper by using a moistened eyelash glued to a wooden dowel. Once mites were on the triangle, it was placed in the whorl of the plant with forceps. Masking tape secured the cage to the conetainer. Populations of WCMs were allowed to build up for approximately one week.

After the mites were on plants for one week, half of the cones were infested with 10 thrips in experiments 1-3 and five thrips in experiment 4. Thrips were collected from alfalfa in experiment 1, volunteer wheat in experiment 2, and grasses in experiments 3 and 4. Thrips were transferred into a small plastic vial with a moistened paintbrush until the vial contained the requisite number of thrips. The vial was placed in the conetainer under the cone cover, the lid of the vial removed, and the cover replaced and secured with masking tape.

Sampling occurred one, two, and three weeks after thrips infestation by randomly selecting four conetainers from each treatment. The number of mites and thrips were counted under a stereo binocular microscope in each sample. Adult thrips were collected and stored in 70% EtOH and later identified to species level. Data were analyzed by using the repeated measures option in PROC MIXED (SAS Institute 2002,2003) to determine differences in mite populations between the two treatments and across sampling dates.

**Effect of thrips on WCM populations in the field.** An estimate of the impact of thrips on WCMs in the field was obtained by determining the difference in WCM numbers

between wheat with different population levels of thrips. In the summer of 2006 and 2008, simulated volunteer winter wheat was planted at the Panhandle Research and Extension Center (PREC) in Scottsbluff, NE. In each experiment, plots were laid out in a randomized complete block design. There were two treatments: half of the plots were treated with an insecticide to control thrips and half of the plots were untreated.

There were four replications in 2006 and individual plots were 2 by 3 m. Half of the plots were treated with acephate at 0.78 l/ha (Orthene, Whitmire Micro-Gen Research Laboratories, Inc.) on 21 August, 2006. Acephate is not detrimental to WCMs but it does control thrips; however, it is not likely to be completely effective (Cote et al. 2002). Prior to insecticide treatment (21 August) and one and two weeks after treatment, one tiller from each of ten randomly selected plants was collected from each plot. Collected samples were placed in a plastic bag, sealed, returned to the laboratory, and stored in the refrigerator. The number of mites and thrips on each tiller was counted under a stereo binocular microscope.

There were eight replications in 2008 and individual plots were 2.5 by 2.5 m. Acephate was applied to half the plots at 0.78 l/ha on 17 July, 2008. Bifenthrin (Capture, FMC Corporation) was applied at 0.44 l/ha 11 days after initial acephate application (28 July) for continued control of thrips. Bifenthrin, like acephate, does not completely eradicate thrips, but is detrimental (Jensen 2001, Reed et al. 2002). Prior to insecticide treatment (16 July) and one, two, three, and five weeks later, two samples of ten plants were randomly collected in each plot. Samples were placed in plastic bags, sealed, and returned to the laboratory. Thrips were immediately extracted from the first sample by

using Berlese funnels and the number of thrips per sample was determined. Plants from the second sample were stored in the refrigerator until inspected. Two tillers from each plant in the second sample were examined under a microscope and the number of mites and thrips was determined. Adult thrips in both samples were collected, stored in 70% ethanol, and identified to species level. Pre-treatment differences between the mean number of WCMs and thrips per tiller and the mean number of thrips per 10 plants were calculated with PROC GLM in SAS (SAS Institute 2003, 2003) in both years. Data collected after initial insecticide treatments were analyzed with the repeated measures option in PROC MIXED (SAS Institute 2002, 2003) to determine differences in WCM and thrips populations between treatments and across sample dates.

**Observation studies.** Nine observation studies were conducted to determine if thrips could be observed consuming WCMs. Black filter paper was placed on the bottom of a round, clear, plastic chamber (ca. 5 cm in diameter and 0.5 cm in depth) and moistened. A wheat tiller obtained from a greenhouse WCM colony that was heavily infested with WCMs was tapped above the filter paper to dislodge mites. The wheat tiller was then placed across the bottom of the observation chamber. One thrips was added to the chamber, and a lid was secured over the filter paper and wheat tiller. The thrips was observed in the chamber for 30 minutes under a microscope.

## Results and Discussion

**Thrips in winter wheat.** A total of 13 thrips species were collected in western Nebraska between 2007 and 2008. In 2007, thrips collections contained four species in Banner County, four species in Cheyenne County, and six species in Scotts Bluff County (Table 3.1). Three species were common to all three counties: *A. obscurus*, *Arorathrips mexicanus* (Crawford), and *Chirothrips aculeatus* Bagnall. In 2008, thrips collections contained 11 species in Banner County and nine species in Cheyenne County. Seven species were common to both counties: *A. obscurus*, *Aeolothrips nasturtii* Jones, *A. mexicanus*, *C. aculeatus*, *F. occidentalis*, *f. tenuicornis*, and *T. tabaci*.

All thrips species collected in 2007 were collected again in 2008. Six additional species were collected in 2008 (Table 3.1). Each sample varied as to the number of thrips species; however, for almost all samples, *A. obscurus* adults were the predominant adult thrips present. Sweep-net samples were more variable with regard to species composition. Seven of the 13 species collected in wheat fields have been reported to be predaceous, indicating that predators are common in winter wheat fields in western Nebraska (Table 3.1). The remaining six species have been reported to feed on grasses and cereal crops or flowers.

Mean thrips larval densities were greater than adult densities per 25 tillers in all samples obtained in 2007 (Appendix E) and the majority of samples in 2008 (Appendix F). The density of larvae was also greater than adults in the majority of sweep-net samples taken in 2008 (Appendix G). In both years, larval and adult populations were highest in mid- to late June; however, extended sampling in 2008 indicated that while

adults increased in number between late May and early July, larval populations began declining between late June and early July as wheat began to senesce. Declining larval populations signal the beginning of thrips dispersal to alternate hosts as they mature and resources decline.

The mean number of thrips per 25 tillers and per 25 sweeps was variable between counties and sampling dates. In 2007, the mean number of thrips per 25 tillers ranged from 1.1 to 7.7 between 5 and 15 June, and between 37.2 and 113.7 between 20 and 27 June. In 2008, the mean number of thrips per 25 tillers ranged from 1.13 to 37.12 between 21 May and 10 June, and between 15.6 and 70.18 between 23 June and 8 July. In 2008, the mean number of thrips per 25 sweeps ranged from 0.25 to 58.75 between 21 May and 10 June, and between 8.25 and 73.75 between 9 and 10 June. The mean number of thrips per 25 sweeps by 7 and 8 July, 2008 had dropped to between 1 and 10.5. In the majority of samples, the mean number of thrips per 25 tillers was highest in late June, while the mean number of thrips per 25 sweeps was highest in mid- to late June (Appendix E, F, G).

In 2007, the mean number of thrips collected per 25 tillers was generally higher in Scotts Bluff County (range: 70.3-80.9; mean: 75.6) when compared to Cheyenne (range: 1.1-113.7; mean: 31.9) and Banner County (range: 4.2-56.0; mean: 26.3) (Fig. 3.1). In 2008, thrips populations per 25 tillers were generally higher in Cheyenne County (range: 9.8-59.0; mean: 29.1) than in Banner County (range: 1.3-70.2; mean: 18.4) (Fig. 3.2). Total mean numbers of thrips per 25 sweeps in 2008 were also generally higher in



Cheyenne County (range: 1.0-58.8; mean: 16.8) than in Banner County (range: 0-73.8; mean: 12.8) (Fig. 3.3).

Because only adults were identified to species, it cannot be assumed that wheat is an acceptable host for all thrips species collected. Winged adults collected in wheat fields may not utilize winter wheat as a host; rather, adults may have been collected inadvertently as they dispersed into wheat fields. Six species were collected in both years and three species were collected from all counties each year, indicating an increased likelihood that those species utilize winter wheat as a host plant. *A. obscurus* was the only species collected in every field that was sampled.

**Effect of thrips on WCM populations in the greenhouse.** The mean number of WCMs per tiller was significantly higher in plants that were not infested with thrips than in plants that were infested with thrips in greenhouse experiment 1 ( $F=10.59$ ;  $df=1,16$ ;  $P=0.005$ ), experiment 2 ( $F=11.30$ ;  $df=1,18$ ;  $P=0.0035$ ), and experiment 3 ( $F=11.81$ ;  $df=1,18$ ;  $P=0.0029$ ), and approached significance in experiment 4 ( $F=4.18$ ;  $df=1,18$ ;  $P=0.0558$ ). Although WCM populations tended to increase with time in both treatments (plants infested with thrips or not infested with thrips), WCM populations tended to increase more rapidly in plants that were not infested with thrips (Figs. 3.4, 3.5, 3.6, and 3.7). The treatment by time interaction was significant in experiment 2 ( $F=4.72$ ;  $df=1,18$ ;  $P=0.0225$ ), and experiment 3 ( $F=8.26$ ;  $df=1,18$ ;  $P=0.0028$ ), indicating WCM populations in plants infested with thrips increased at a lower rate than those without thrips.

The mean number of thrips in infested plants was variable over weeks and experiments; however, thrips populations tended to increase with time (Table 3.2).

Thrips larvae were collected from plants more frequently than adults. Adults collected from greenhouse plants were identified to species. *A. obscurus* and *T. tabaci* were collected in experiment 1, *A. obscurus*, *Aptinothrips stylifer* Tryborn, and *Aptinothrips rufus* Haliday were collected in experiment 2, and *A. obscurus*, *A. stylifer*, *A. rufus*, and *F. occidentalis* were collected in experiments 3 and 4.

Some thrips were discovered on plants that were not infested with thrips. More thrips were discovered on uninfested plants in experiment 1 (range: 1-31) than on uninfested plants in the other three experiments (range: 1-8). More precautions were taken in later experiments to decrease the likelihood of thrips invading uninfested plants. Cages were secured with masking tape to containers in later experiments immediately after wheat was planted rather than after plants began growing. The ability of thrips to invade plants may have contributed to variation in subsequent WCM numbers in plants that were not infested with thrips, especially in experiment 1 (see Fig. 3.4). Although thrips were discovered on plants not infested with thrips, thrips populations were significantly higher in plants infested with thrips than those plants not infested with thrips in all four experiments (experiment 1:  $F=10.40$ ;  $df=1,16$ ;  $P=0.0053$ ; experiment 2:  $F=56.93$ ;  $df=1,18$ ;  $P<0.0001$ ; experiment 3:  $F=10.79$ ;  $df=1,18$ ;  $P=0.0041$ ; experiment 4:  $F=9.41$ ;  $df=1,18$ ;  $P=0.0066$ ).

WCM densities over experiments were variable. In experiment 1, WCM populations in plants that were not infested with thrips increased between week one and week two, but decreased between week two and week three (see Fig. 3.4). Visible differences in plant condition appeared by week three of experiment 1. Plants not

infested with thrips were brown and dry with very little green plant material while plants infested with thrips were mostly green and visibly healthier. The mean number of WCMs on plants not infested with thrips decreased by nearly half between sampling at two weeks (14,637 WCMs) and sampling at three weeks (7,559 WCMs). Because mite infested plants in week three had begun to dry out, it is likely that mite numbers began to diminish as food and habitat quality were reduced. The mean number of WCMs on plants infested with thrips didn't differ greatly between week two (1,225 WCMs) and week three (958 WCMs), indicating thrips were impacting mite populations.

Plants not infested with thrips in experiments 2-4 did not suffer as much damage as plants in experiment 1. However, plants infested with thrips in experiments 2-4 had light to heavy thrips feeding damage. Thrips feeding caused plants to be drier, and plant damage from thrips feeding may have decreased the ability of WCMs to survive and reproduce.

In all greenhouse experiments, the number of WCMs per plant was highly variable within replications. Female WCMs lay approximately 12 eggs, and the complete cycle from egg to egg typically takes 8-10 days (Staples and Allington 1956). Large populations of mites can build up quickly, and high reproduction rates can result in variability in population size.

Thrips species utilized in experiments were variable and the species of thrips utilized to infest plants could not be determined prior to infesting plants. The majority of thrips observed on greenhouse plants were larvae and were not identified to species level. Thrips adults collected in these studies that have been reported to consume insects or

mites were *T. tabaci*, *F. occidentalis*, and *A. obscurus* (Wilson et al. 1996, Milne and Walter 1998, Trichilo and Leigh 1986, Thompson and Simmonds 1964). It is likely that the same species of thrips were not utilized to infest all replications. If a mix of herbivorous and predaceous thrips infested on the plants varied, the impact on WCM populations would be variable.

**Effect of thrips on WCM populations in the field.** In 2006, the mean number of mites per tiller in pre-treatment samples was not significantly different between treated and untreated plots. Densities of WCMs in the field were numerically higher in plots where thrips were controlled than in untreated plots one and two weeks after treatment (Fig. 3.8). Differences between WCM populations in treated and untreated plots were analyzed with repeated measures. The number of WCMs in plots treated with insecticide to control thrips were significantly higher than in untreated plots ( $F=13.98$ ;  $df=1,12$ ;  $P=0.0028$ ).

Thrips populations in 2006 were significantly higher in untreated plots compared to treated plots prior to insecticide treatment ( $F$  value= $31.78$ ;  $df=1,3$ ;  $P=0.01$ ). Thrips populations in both treated and untreated plots decreased between samples taken prior to treatment and samples taken one week after treatment (Fig. 3.9). Thrips populations increased between one and two weeks after treatment in both treated and untreated plots; however, thrips populations were significantly higher in untreated plots when compared to treated plots ( $F=15.78$ ;  $df=1,12$ ;  $P=0.0019$ ).

In 2008, the mean number of WCMs was not significantly different between treated and untreated plots for samples taken prior to treatment. WCM populations were

significantly higher in untreated plots compared to plots treated with insecticide to control thrips when analyzed with repeated measures ( $F=60.76$ ;  $df=1,56$ ;  $P<0.0001$ ). Although WCM populations increased over time in both treatments, WCM populations were higher in untreated plots compared to treated plots (Fig. 3.10). The treatment by time interaction was significant ( $F=13.05$ ;  $df=1,56$ ;  $P<0.0001$ ), indicating WCM populations in untreated plots increased at a greater rate.

The mean number of thrips per tiller was not significantly different prior to insecticide treatment in the first experiment; therefore, the number of replications was increased in the second experiment to enhance the likelihood of determining differences in thrips populations between treatments. The number of plant samples used to determine thrips presence was also increased by determining thrips populations in 10 plants per plot to increase the likelihood of detecting differences in thrips populations between treatments.

The mean number of thrips per tiller and the mean number of thrips per 10 plants were not significantly different between treatments prior to treatment. The mean number of thrips per tiller tended to decrease between samples taken prior to treatment and samples taken one and two weeks after treatment, and increased between two and five weeks (Fig. 3.11); however, thrips populations were significantly higher in untreated plots when compared to treated plots according to repeated measures analysis ( $F=6.39$ ;  $df=1,56$ ;  $P=0.0143$ ). A similar trend occurred when analyzing differences between treatments in the number of thrips per 10 plants (Fig. 3.12). Mean thrips populations per 10 plants were not significantly different prior to insecticide treatment; however, across

sampling dates thrips populations per 10 plants were significantly higher in untreated plots when compared to treated plots ( $F=5.66$ ;  $df=1,56$ ;  $P=0.0208$ ).

The results of field studies indicate thrips have an effect on mite populations. Although WCM populations increased weekly for both untreated and treated plots, the mean number of mites in plots treated with insecticide increased more rapidly than the number of mites in untreated plots, indicating thrips presence had a negative impact on mite populations.

In 2006, thrips adults were not collected for thrips identification. In 2008, thrips larvae were collected more frequently than adults. Adults collected from both treated and untreated plots were identified as *A. obscurus*, *A. nasturtii*, *F. schultzei*, and *F. tenuicornis*. *T. tabaci* was only collected from untreated plots and *A. mexicanus* was only collected from treated plots. *A. nasturtii*, *F. schultzei*, *T. tabaci*, and *A. obscurus* have been reported to be predaceous on mites or insects (Hoddle et al. 2004, Wilson et al. 1996, Milne and Walter 1997, Milne and Walter 1998).

**Observation studies.** In six of nine observation studies, thrips were not observed to consume WCMs or even appear to be distinctly aware of them. Thrips appeared to be searching for escape from their enclosure. Their movement was rapid concentrated on the edges of the enclosure. Often, mites would adhere to the legs, antennae or body of the thrips and the thrips would continue to walk until the mite became dislodged. Occasionally, thrips attempted to dislodge mites that had become adhered to them.

The first observation of feeding was by a tan thrips larvae, later identified as *F. occidentalis*, obtained from grasses at PREC in Scottsbluff, NE, and starved for 24 hours.

Within five minutes of the beginning of the observation, a WCM moving on the bottom of the cage bumped into the foreleg of the thrips. The thrips stretched out its leg and pulled the mite underneath its body and mouthparts. The mite shrank in size as the thrips stood over it. While the thrips was feeding on the first mite, a second mite bumped into the thrips foreleg; the thrips extended its leg, pulled the mite closer to its body, and held it trapped under its tarsus while it fed. The mite escaped before the thrips finished feeding on the first mite. After approximately 1-2 minutes, the thrips moved away from the mite it was feeding on, and a white shriveled skin was observed where the mite had been. During this observation, the thrips consumed a total of three mites in 20 minutes. Within three minutes of feeding, the thrips appeared to clean its antennae and mouthparts on the bottom of the enclosure.

The second observation of a thrips feeding on WCMs was by a light brown adult obtained from a greenhouse colony of mites (identified as *T. tabaci*). The thrips was not starved prior to the experiment. After 20 minutes in the observation enclosure, the thrips trapped a mite under its left front leg, and the mite was observed to shrink as the thrips fed. When the thrips moved away from the mite, a shriveled white ball was left where the mite had been. The thrips did not appear to be searching for prey. After feeding, the thrips appeared to clean itself as described above. After approximately five minutes, the thrips consumed another WCM in a similar fashion. Two mites were consumed in 30 minutes and feeding on each mite lasted approximately 30 seconds to two minutes.

In the third observation, a larval thrips that could not be identified appeared to be feeding on WCMs. The thrips used in this study was a yellow tube-shaped larva obtained from a mixed colony of mites in the greenhouse. The thrips was not starved prior to the

experiment. During the first 20 minutes of the experiment, various mites crawled on the thrips, and the thrips appeared irritated by this behavior. The thrips walked over many mites without appearing to notice them. After 20 minutes, the thrips stopped over a cluster of 6-10 mites. The thrips appeared to pull the grouping of mites towards itself. The thrips stayed over the mites for approximately five minutes. It appeared the thrips consumed some mites; however, because there was a large cluster of mites (6-10), it was difficult confirm the exact behavior. The thrips moved away from the cluster of mites and stopped over a solitary mite at the edge of the enclosed chamber for approximately one minute. Although it appeared to be feeding, the mite was located at the edge of the chamber near the sealed lid and a shriveled skin was not observed.

Observation studies clearly indicate that at least some thrips species will eat WCMs. The adult specimen that was observed to consume mites was *T. tabaci* and the larva was *F. occidentalis*. The *T. tabaci* adult had not been starved prior to the experiment; rather, it was collected from a wheat plant in the greenhouse that served as host for a mixed colony of WCMs. The three thrips observed to feed on WCMs did not appear to actively search for mites, but rather feeding occurred after apparent inadvertent contact.



## Conclusions

Although the majority of Thysanoptera are associated with green plants or fungi, obligate predators exist, and facultative predation is widespread (Mound 2002). Of the 18 adult species collected in samples for species diversity in winter wheat, field and greenhouse trials on the impact of thrips on WCMs, and observation studies, 11 are reported to be either obligate or facultative predators. The remaining species are associated with grasses and cereal crops or flowers.

Many predaceous species were collected in these studies; however, not all predaceous species were present in each experiment and the distribution of predaceous species varied widely between and among experiments. Species reported to be predaceous other than the two species observed to consume WCMs (*T. tabaci* and *F. occidentalis*) may or may not be predaceous on WCMs. Studies indicate that many species reported to be predaceous do not appear to be specifically adapted to predation but that predation may be the result of limited protein sources from primary host plants or the coincidental presence of prey (Trichilio and Leigh 1988, Milne and Walter 1997, 1998). This may explain why variation in experiments occurred throughout the studies.

Results of both field and greenhouse experiments indicate that thrips limit WCM populations. Knowledge of this phenomenon may be critical to researchers that aim to utilize WCM populations in experiments. Researchers that conduct studies where WCM populations are needed, such as plant resistance trials, should be aware of the impact of thrips on WCMs. Thrips may need to be controlled in these situations.

Thrips populations can impact the buildup of populations of WCMs. WCMs utilize pre-harvest volunteer wheat as a green-bridge host between summer harvest and the emergence of the fall crop (Gibson and Painter 1956, Staples and Allington 1956). These studies indicate that thrips do have a regulating effect on mite populations, but these effects are not likely great enough to eliminate the threat of virus spread from mite populations.

Studies designed to explore the relationship between a particular species of thrips such as *T. tabaci* or *F. occidentalis* and WCM populations would supplement our knowledge on thrips predation on WCMs and its impact. Additional studies should focus on determining if other species of thrips feed on WCMs and determining the impact of thrips on mites in volunteer wheat.

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## Tables

Table 3.1. Species of adult thrips collected in 2007 and 2008 by location.

Location	2007 Species Present	2008 Species Present
Banner County	<i>Anaphothrips obscurus</i> (Muller)* <i>Arorathrips mexicanus</i> (Crawford) <i>Chirothrips aculeatus</i> Bagnall <i>Chirothrips falsus</i> Priesner	<i>Aeolothrips fasciatus</i> (L.)* <i>Aeolothrips nasturtii</i> Jones* <i>Anaphothrips obscurus</i> (Muller)* <i>Arorathrips mexicanus</i> (Crawford) <i>Chirothrips aculeatus</i> Bagnall <i>Chirothrips falsus</i> Priesner <i>Chirothrips manicatus</i> Haliday <i>Frankliniella occidentalis</i> (Pergande)* <i>Frankliniella tenuicornis</i> (Uzel)* <i>Thrips simplex</i> (Morison) <i>Thrips tabaci</i> Lindeman*
Cheyenne County	<i>Anaphothrips obscurus</i> (Muller)* <i>Arorathrips mexicanus</i> (Crawford) <i>Chirothrips aculeatus</i> Bagnall <i>Frankliniella tenuicornis</i> (Uzel)*	<i>Aeolothrips nasturtii</i> Jones* <i>Anaphothrips obscurus</i> (Muller)* <i>Arorathrips mexicanus</i> (Crawford) <i>Chirothrips aculeatus</i> Bagnall <i>Frankliniella fusca</i> (Hinds)* <i>Frankliniella occidentalis</i> (Pergande)* <i>Frankliniella tenuicornis</i> (Uzel)* <i>Franklinothrips vespiformis</i> (Crawford)* <i>Thrips tabaci</i> Lindeman*
Scotts Bluff County	<i>Anaphothrips obscurus</i> (Muller)* <i>Arorathrips mexicanus</i> (Crawford) <i>Chirothrips aculeatus</i> Bagnall <i>Chirothrips falsus</i> Priesner <i>Thrips simplex</i> (Morison) <i>Thrips tabaci</i> Lindeman*	

\* Species reported as predaceous.



Table 3.2. Mean  $\pm$  standard error (SE) number of thrips collected from greenhouse plants infested with thrips (n=4). Initial infest: experiments 1-3: 10 wheat curl mite (WCM) and 10 thrips per conetainer; experiment 4: 5 WCMs and 5 thrips per conetainer.

	Mean $\pm$ SE (range)		
	Week 1	Week 2	Week 3
<b>Experiment 1 (2007)</b>	18.75 $\pm$ 1.18 (17-22)	12.25 $\pm$ 8.94 (2-39)	52.25 $\pm$ 15.99 (26-96)
<b>Experiment 2 (2008)</b>	26.50 $\pm$ 6.73 (8-37)	117.50 $\pm$ 15.89 (89-146)	51.50 $\pm$ 18.53 (14-98)
<b>Experiment 3 (2009)</b>	5.5 $\pm$ 4.84 (0-20)	29.75 $\pm$ 10.99 (2-53)	28.0 $\pm$ 9.91 (13-56)
<b>Experiment 4 (2009)</b>	0	34.25 $\pm$ 8.27 (18-49)	41.25 $\pm$ 22.63 (10-108)

## Figures

Fig. 3.1. Mean number of thrips per 25 tillers by date in each field and county (Banner County-BC; Cheyenne County-CC; Scotts Bluff County-SBC) in 2007 (\*not sampled in early June). Standard error bars are shown.

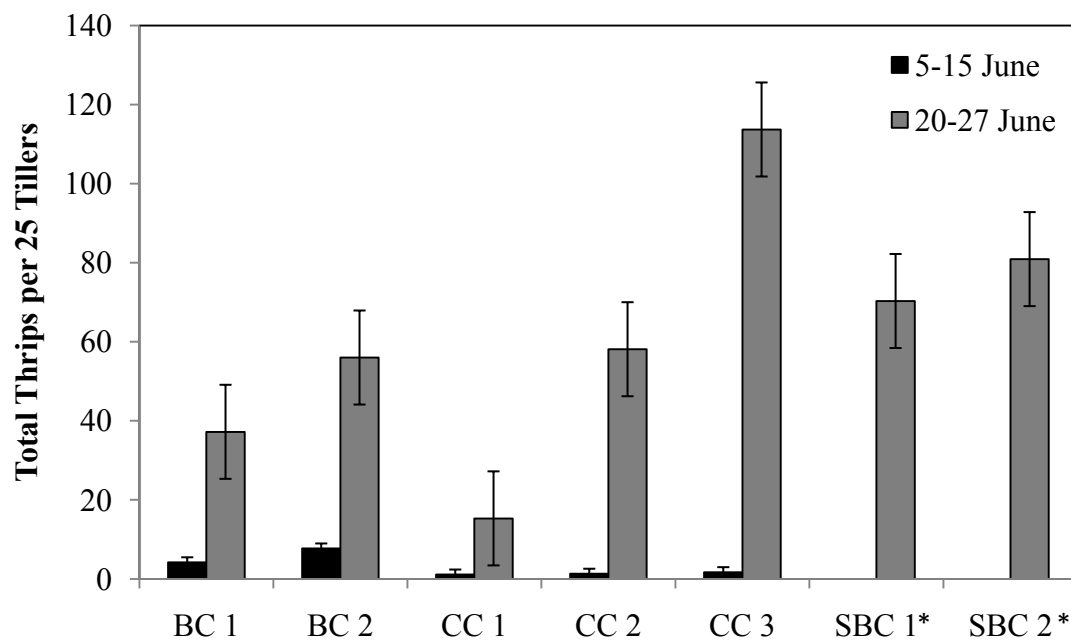


Fig. 3.2. Mean number of thrips per 25 tillers by date in each field and county (Banner County-BC; Cheyenne County-CC) in 2008. Standard error bars are shown.

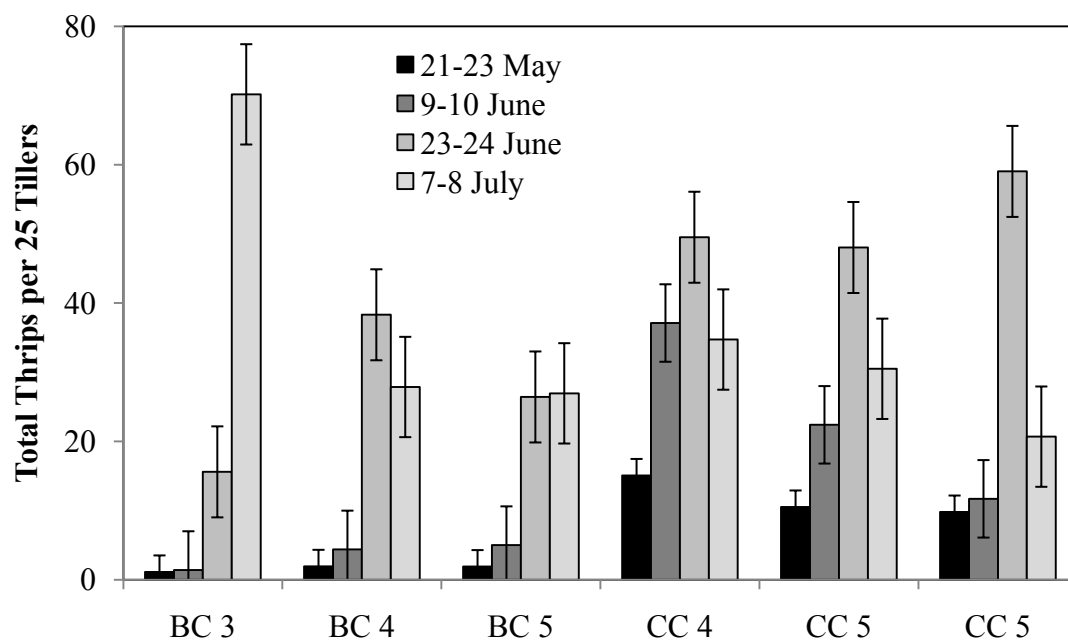


Fig. 3.3. Mean number of thrips per 25 sweeps by date in each field and county (Banner County-BC; Cheyenne County-CC ) in 2008 (\*not sampled in May). Standard error bars are shown.

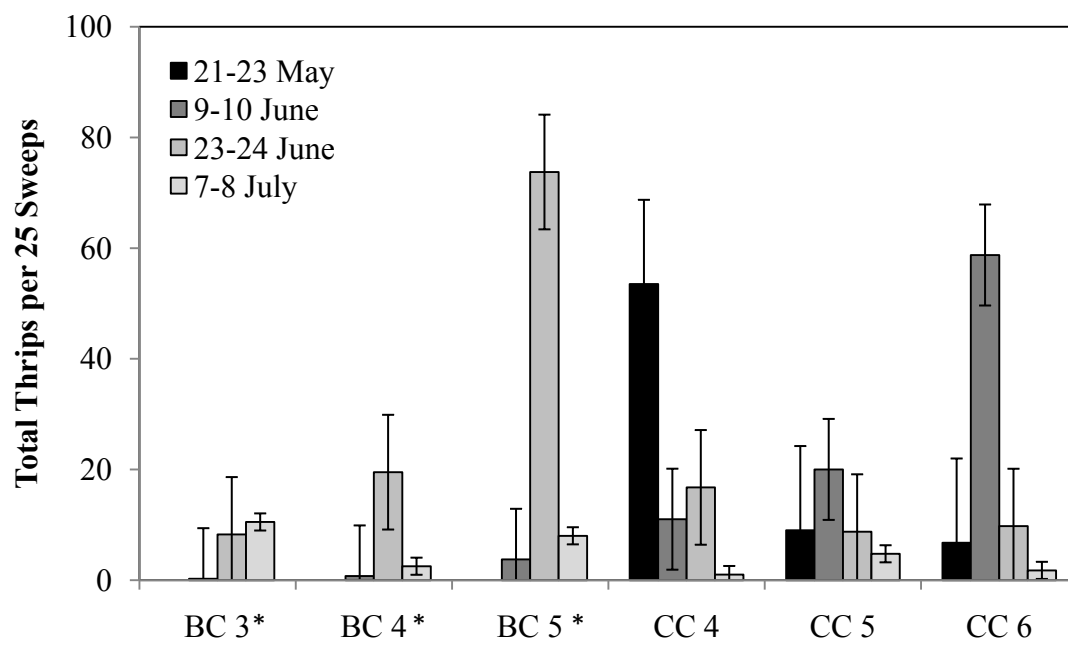


Fig. 3.4. Mean number of wheat curl mites (WCM) per conetainer in greenhouse plants infested with thrips and greenhouse plants not infested with thrips in experiment 1. Standard error bars are shown in red.

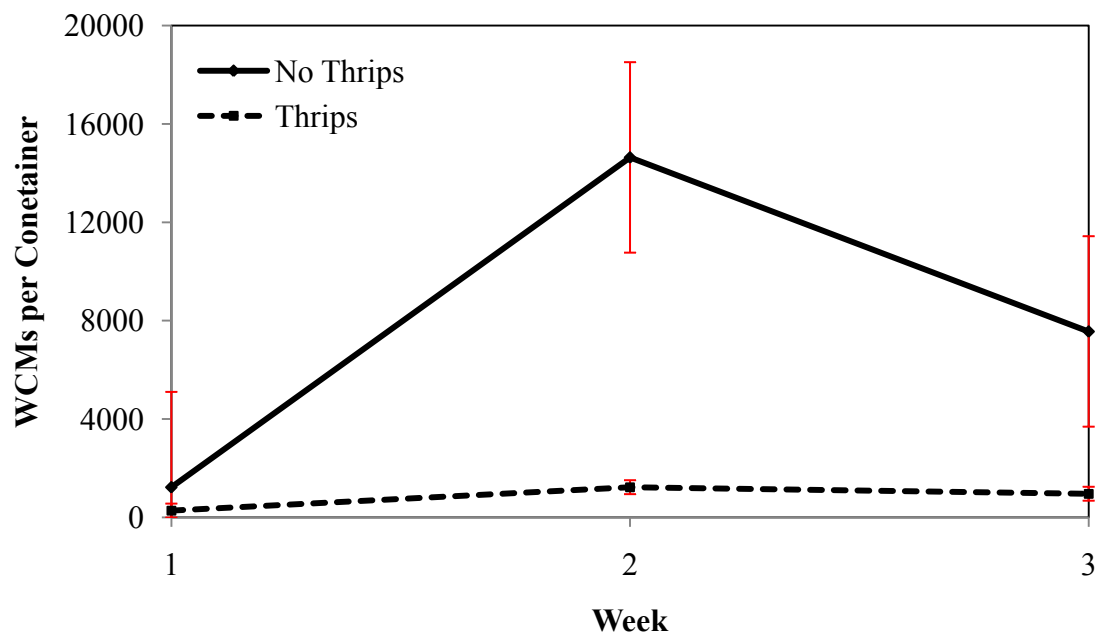


Fig. 3.5. Mean number of wheat curl mites (WCM) per conetainer in greenhouse plants infested with thrips and greenhouse plants not infested with thrips in experiment 2. Standard error bars are shown in red.

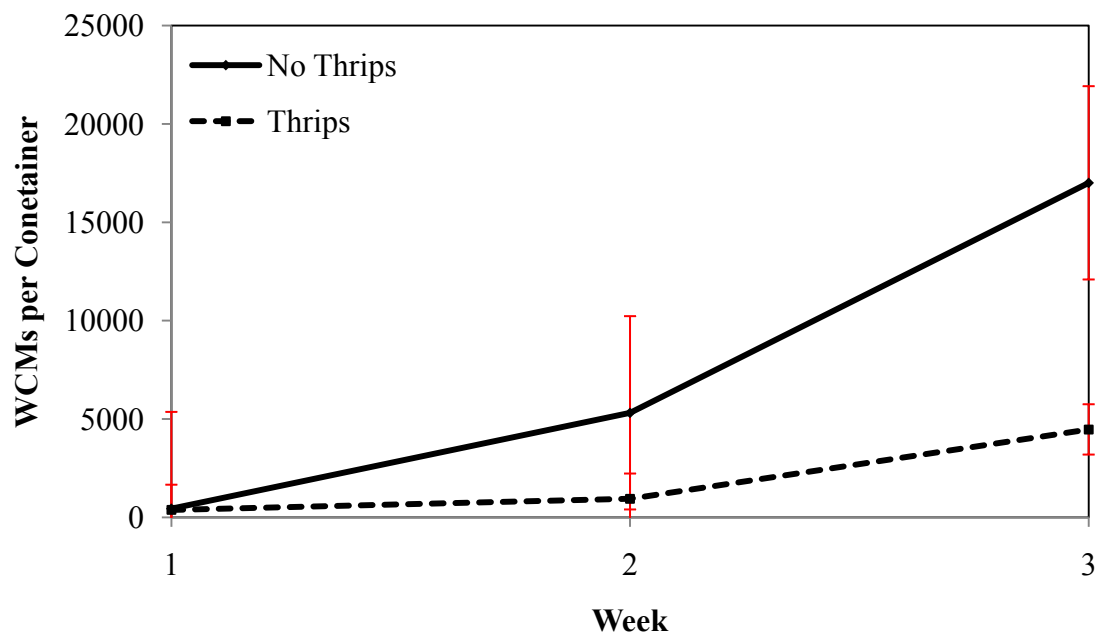


Fig. 3.6. Mean number of wheat curl mites (WCM) per conetainer in greenhouse plants infested with thrips and greenhouse plants not infested with thrips in experiment 3. Standard error bars are shown in red.

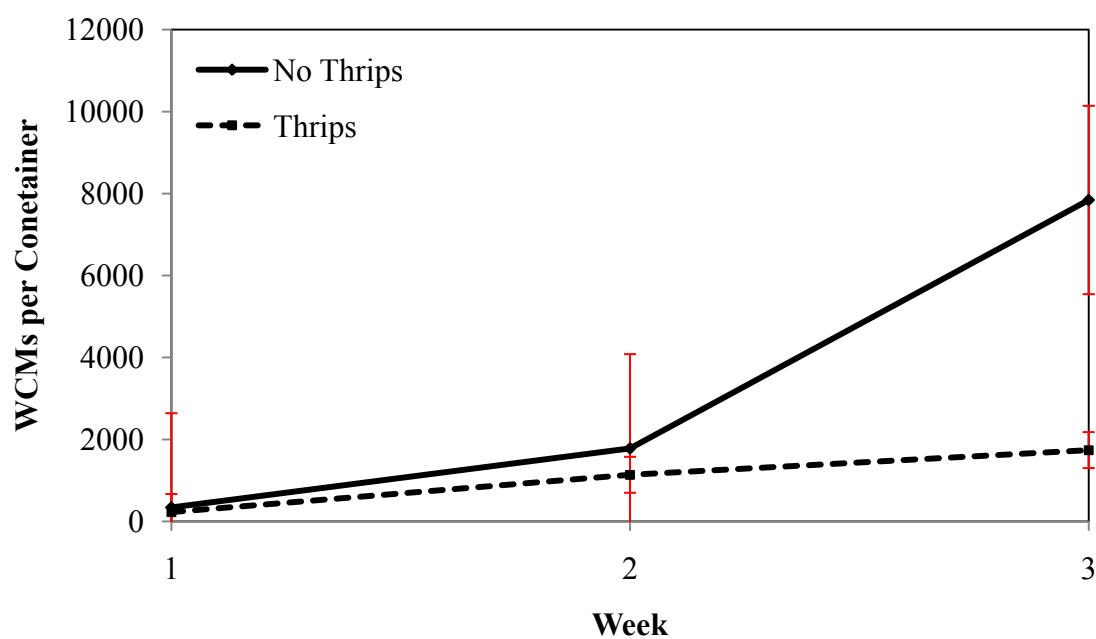




Fig. 3.7. Mean number of wheat curl mites (WCM) per conetainer in greenhouse plants infested with thrips and greenhouse plants not infested with thrips in experiment 4. Standard error bars are shown in red.

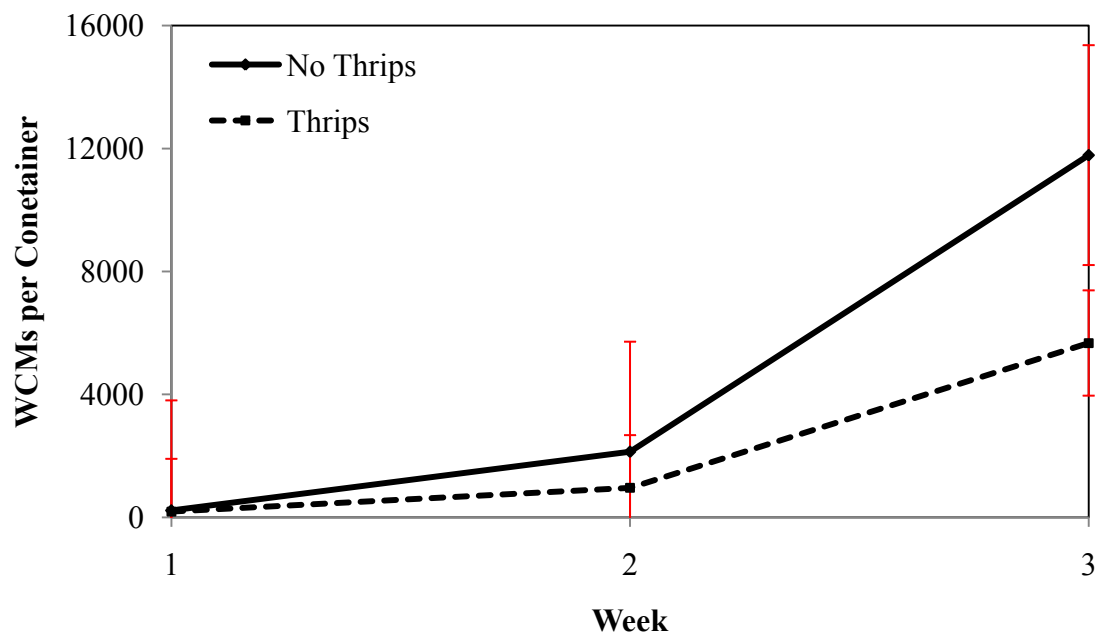


Fig. 3.8. Mean number of wheat curl mites (WCM) per tiller in plots treated with insecticide to control thrips (Treated) and untreated (Untreated) plots in 2006. Week 0 denotes samples taken prior to treatment. Standard error bars are shown in red.

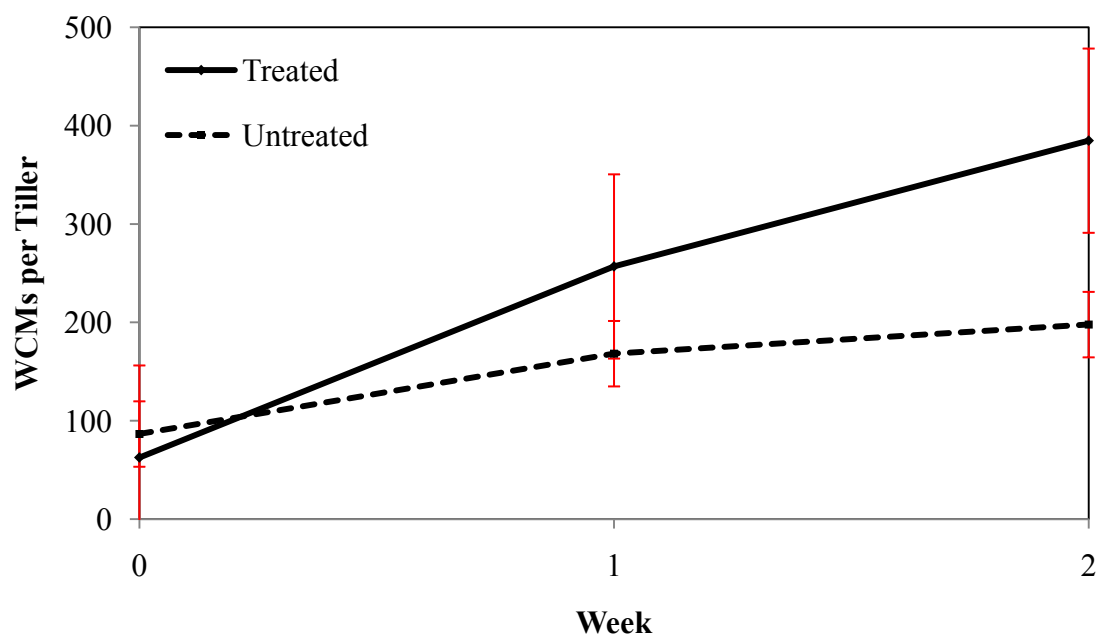


Fig. 3.9. Mean number of thrips per tiller in plots treated with insecticide to control thrips (Treated) and untreated (Untreated) plots in 2006. Week 0 denotes samples taken prior to treatment. Standard error bars are shown in red.

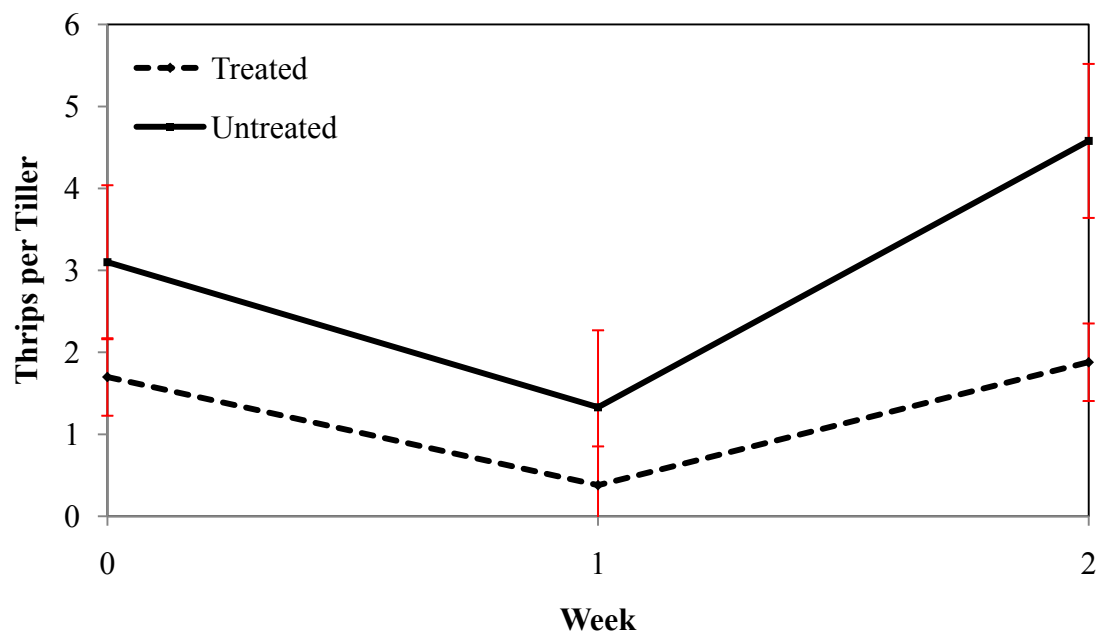


Fig. 3.10. Mean number of wheat curl mites (WCM) per tiller in plots treated with insecticide to control thrips (Treated) and untreated (Untreated) plots in 2008. Week 0 denotes samples taken prior to treatment (\*No data was taken four weeks after initial insecticide treatment). Standard error bars are shown in red.

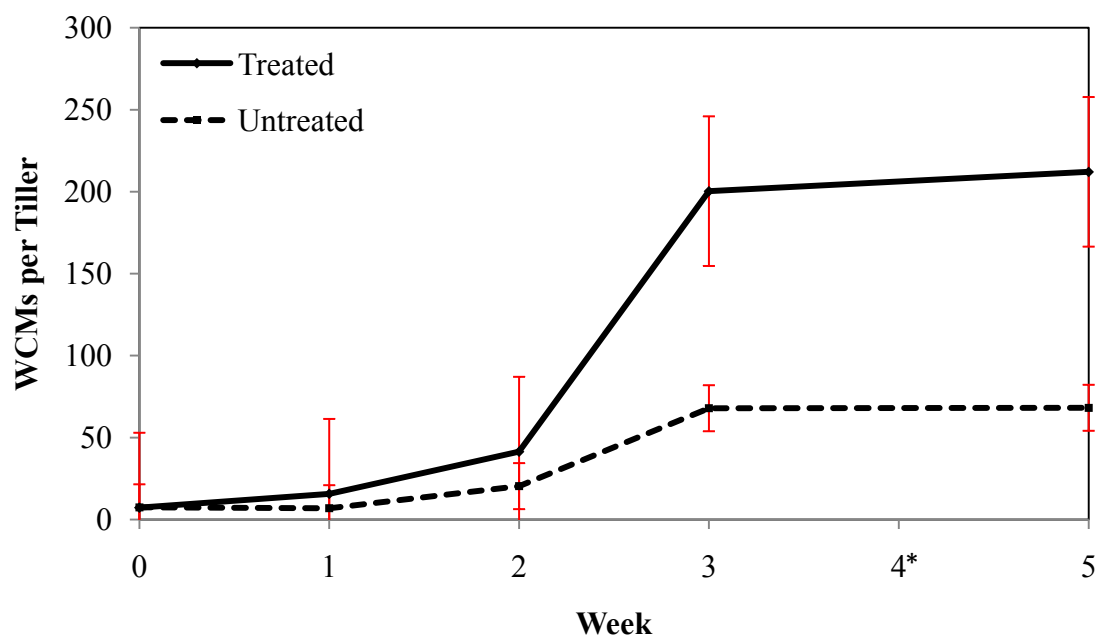


Fig. 3.11. Mean number of thrips per tiller in plots treated with insecticide to control thrips (Treated) and untreated (Untreated) plots in 2008. Week 0 denotes samples taken prior to treatment (\*No data was taken four weeks after initial insecticide treatment). Standard error bars are shown in red.

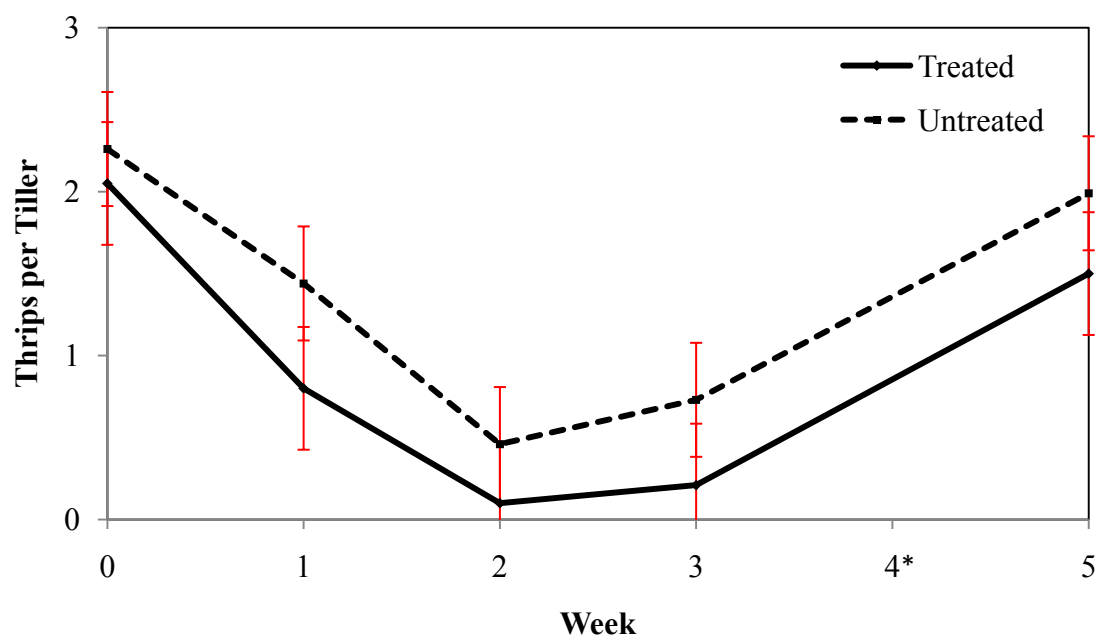
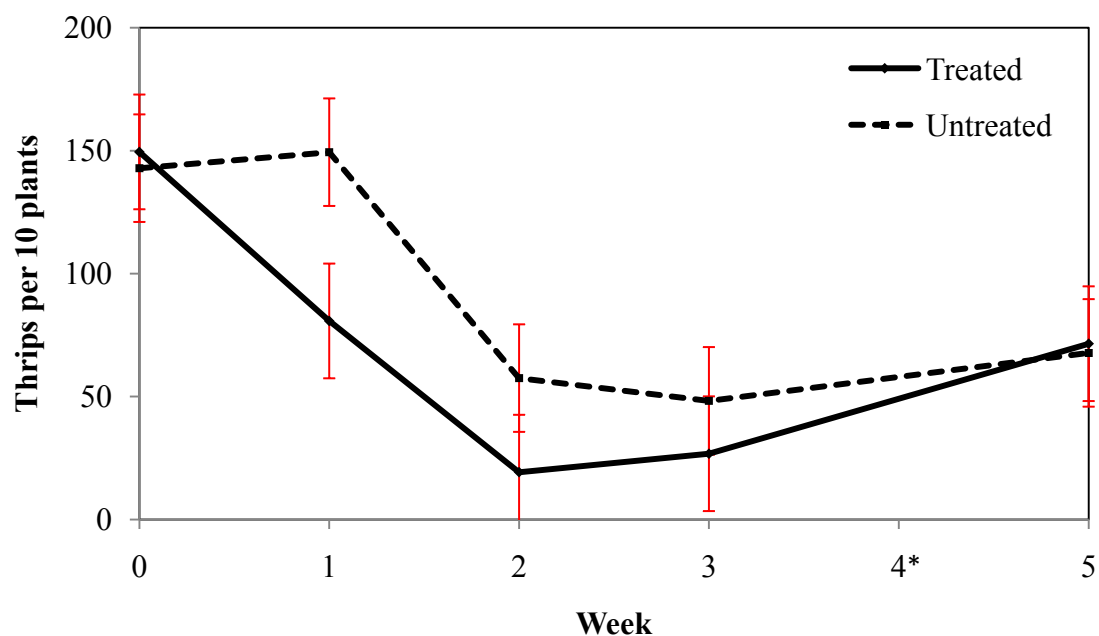


Fig. 3.12. Mean number of thrips per 10 plants in plots treated with insecticide to control thrips (Treated) and untreated (Untreated) plots in 2008. Week 0 denotes samples taken prior to treatment (\*No data was taken four weeks after initial insecticide treatment). Standard error bars are shown in red.



Appendix A: Volunteer wheat plot.



Appendix A. Collection of hyperspectral data (image: Art Zygielbaum)





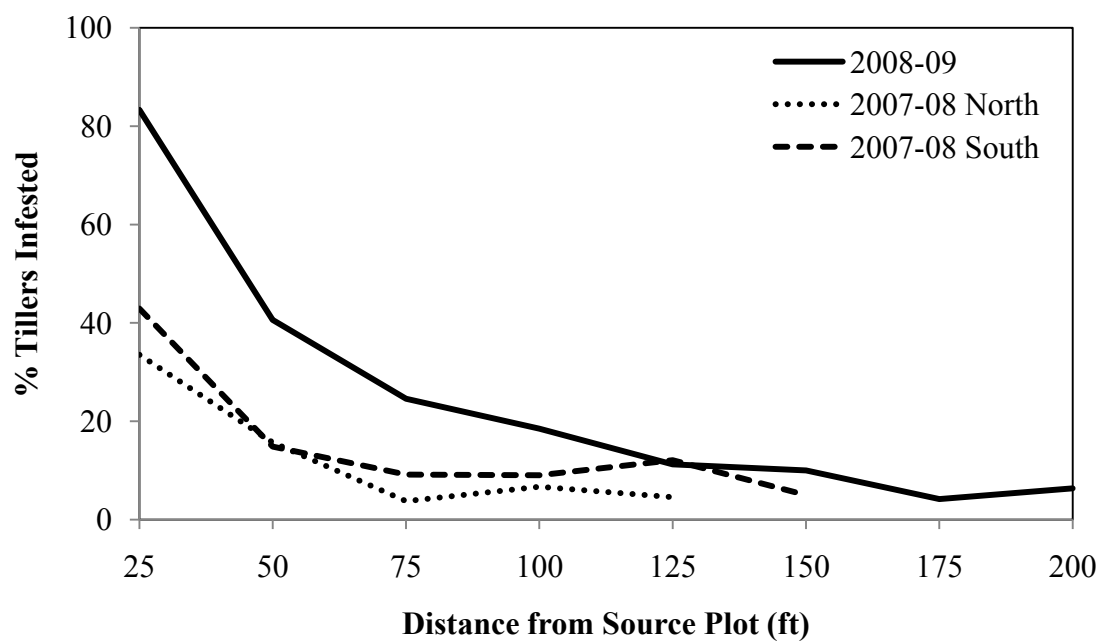
Appendix A. Ocean Optics backpack set-up (image: Art Zygielbaum).



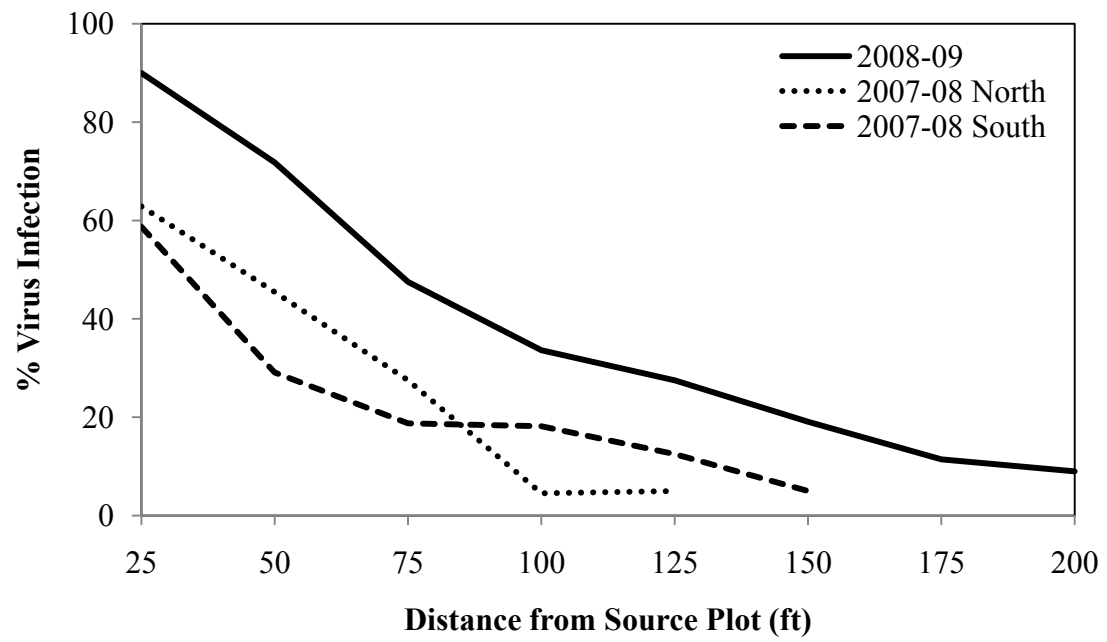
Appendix A. Virus symptoms spread from the wheat curl mite source volunteer plot in the spring of 2009.



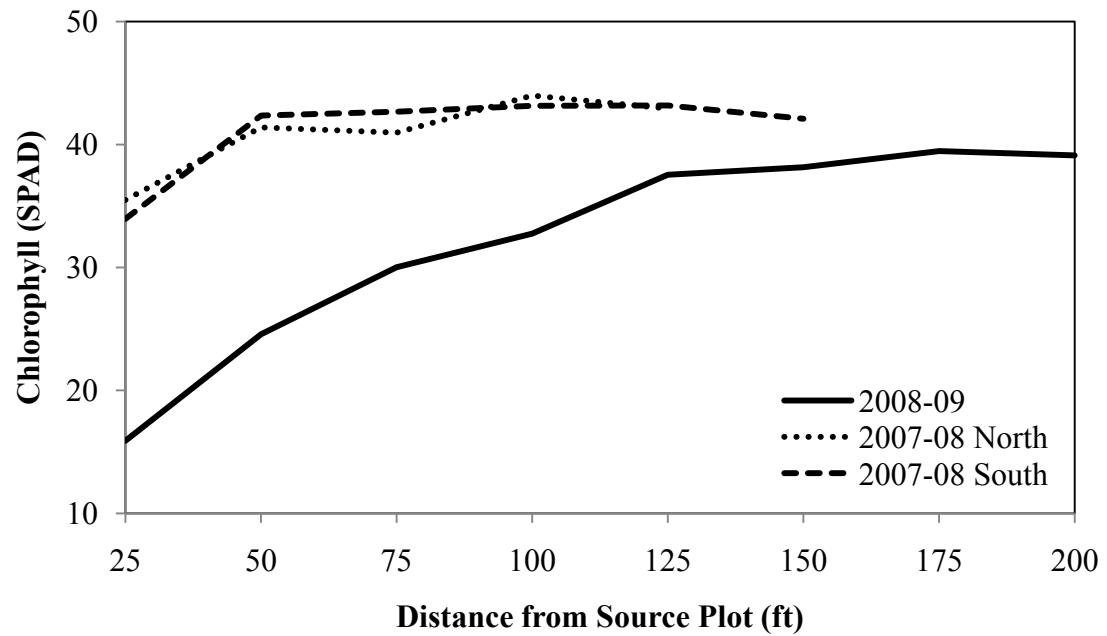
Appendix B: Mean percent of wheat tillers in each sampling ring infested with wheat curl mites for each year and plot.



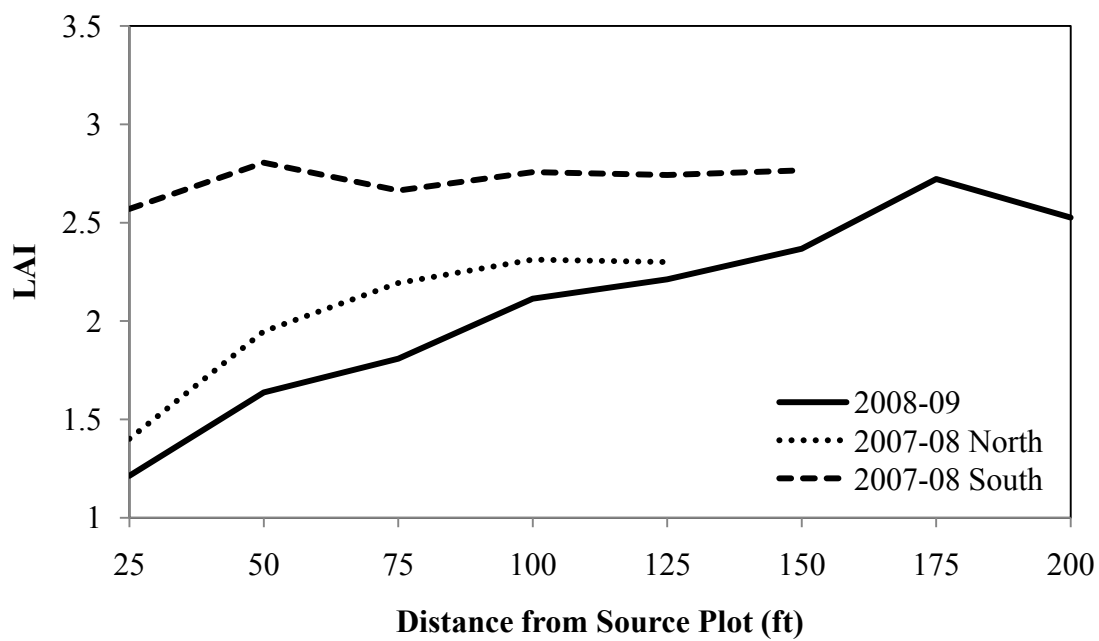
Appendix B: Mean percent virus infection in each sampling ring for each year and plot.



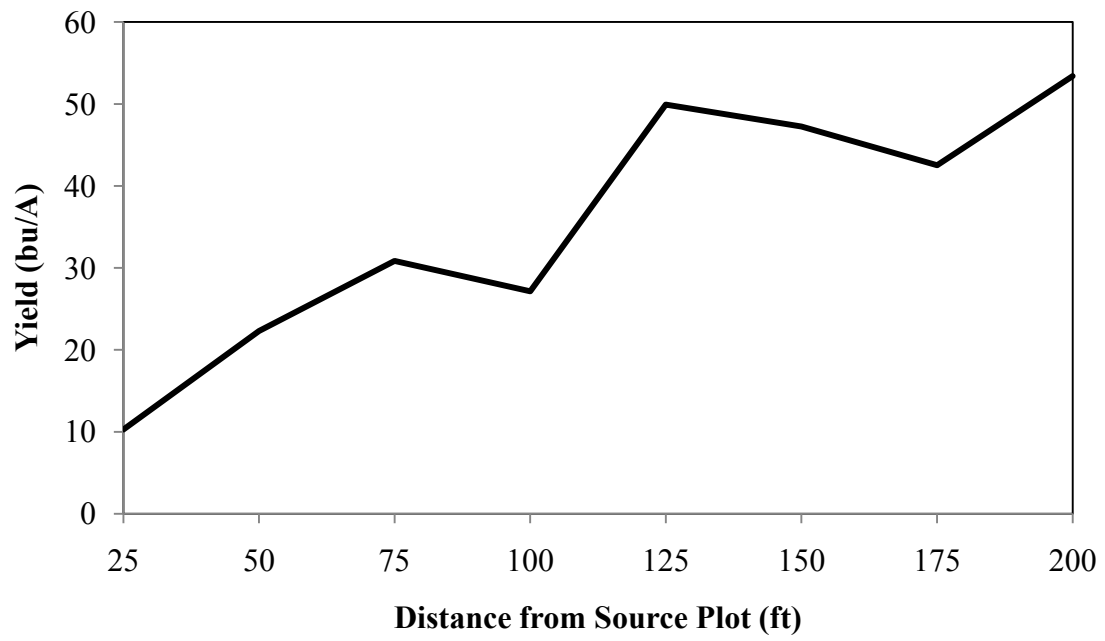
Appendix B: Mean relative chlorophyll content in each sampling ring for each year and plot.



Appendix B: Mean leaf area index (LAI) in each sampling ring for each year and plot.



Appendix B: Mean yield in each sampling ring in 2008-09.





Appendix C: 2007-08 north plot. Camera is pointed southeast.





Appendix C: 2007-08 south plot. Camera is pointed southeast.



Appendix C: 2008-09 plot. Camera is pointed southeast.



Appendix D: Number of hours wind originated from each direction between 27  
September and 30 November.

Direction	2006-07	2007-08	2008-09
North (332.5°-0°; 0°-22.5°)	226	530	166
Northeast (22.5°-67.5°)	38	66	35
East (67.5°-112.5°)	72	32	72
Southeast (112.5-157.5°)	130	52	217
South (157.5°-202.5°)	181	132	78
Southwest (202.5°-247.5°)	224	184	129
West (247.5°-292.5°)	370	326	395
Northwest (292.5°-332.5°)	305	232	358

Appendix E: Mean  $\pm$  standard error (SE) number of thrips adults, larvae, and total thrips per 25 wheat tillers in 2007.

Field (Crop Rotation)	Date	Mean $\pm$ SE (range)		
		Thrips Adults	Thrips Larvae	Total Thrips
Banner County 1 (Wheat-Fallow)	06/15/2007	0.40 $\pm$ 0.27 (0-2)	3.80 $\pm$ 0.84 (1-9)	4.20 $\pm$ 0.79 (1-9)
	06/27/2007	11.00 $\pm$ 2.88 (4-28)	26.20 $\pm$ 4.78 (8-53)	37.20 $\pm$ 7.39 (14-78)
Banner County 2 (Wheat-Fallow)	06/15/2007	0.90 $\pm$ 0.23 (0-2)	6.80 $\pm$ 0.94 (3-11)	7.70 $\pm$ 1.10 (3-13)
	06/27/2007	12.60 $\pm$ 3.87 (0-36)	43.40 $\pm$ 30.47 (0-316)	56.0 $\pm$ 30.69 (0-325)
Cheyenne County 1 (Wheat-Sunflower- Millet-Fallow)	06/05/2007	0.20 $\pm$ 0.13 (0-1)	0.90 $\pm$ 0.41 (0-4)	1.10 $\pm$ 0.43 (0-4)
	06/26/2007	5.10 $\pm$ 1.11 (0-13)	10.20 $\pm$ 4.38 (2-49)	15.30 $\pm$ 4.46 (6-53)
Cheyenne County 2 (Wheat-Fallow)	06/05/2007	0.40 $\pm$ 0.16 (0-1)	0.90 $\pm$ 0.69 (0-7)	1.30 $\pm$ 0.67 (0-7)
	06/26/2007	3.50 $\pm$ 0.83 (0-8)	54.6 $\pm$ 19.24 (11-206)	58.10 $\pm$ 18.77 (15-206)
Cheyenne County 3 (Wheat-Corn-Fallow)	06/05/2007	0.40 $\pm$ 0.22 (0-2)	1.30 $\pm$ 1.34 (0-4)	1.70 $\pm$ 1.34 (0-4)
	06/26/2007	34.00 $\pm$ 17.14 (3-180)	79.70 $\pm$ 31.90 (9-288)	113.70 $\pm$ 45.92 (3-418)
Scotts Bluff County 1 (Wheat-Fallow)	06/20/2007	24.90 $\pm$ 9.88 (10-113)	45.40 $\pm$ 6.33 (13-69)	70.30 $\pm$ 7.95 (32-126)
Scotts Bluff County 2 (Wheat-Fallow)	06/20/2007	12.70 $\pm$ 2.18 (5-26)	68.20 $\pm$ 10.45 (35-151)	80.90 $\pm$ 12.27 (40-177)

Appendix F: Mean  $\pm$  standard error (SE) number of thrips adults, larvae, and total thrips per 25 wheat tillers in 2008.

Field (Crop Rotation)	Date	Mean $\pm$ SE (range)		
		Thrips Adults	Thrips Larvae	Total Thrips
Banner County 3 (Wheat-Fallow)	05/23/2008	1.09 $\pm$ 0.21 (0-2.27)	0.22 $\pm$ 0.09 (0-0.71)	1.31 $\pm$ 0.23 (0-2.27)
	06/10/2008	0.69 $\pm$ 0.23 (0-2.24)	0.73 $\pm$ 0.22 (0-2.17)	1.41 $\pm$ 0.36 (0-3.26)
	06/23/2008	4.23 $\pm$ 0.63 (0.68-7.69)	11.37 $\pm$ 3.02 (2.73-32.41)	15.60 $\pm$ 3.32 (5.91-38.89)
	07/07/2008	19.15 $\pm$ 3.42 (2.54-41.67)	51.03 $\pm$ 11.42 (7.20-118.02)	70.18 $\pm$ 12.60 (9.75-131.40)
Banner County 4 (Wheat-Fallow)	05/23/2008	0.29 $\pm$ 0.08 (0-0.88)	1.65 $\pm$ 0.54 (0.22-5.61)	1.94 $\pm$ 0.53 (0.49-5.61)
	06/10/2008	1.21 $\pm$ 0.23 (0.31-2.50)	3.18 $\pm$ 0.50 (1.38-6.16)	4.39 $\pm$ 0.60 (1.83-7.61)
	06/23/2008	1.38 $\pm$ 0.43 (0-5.16)	36.93 $\pm$ 6.28 (4.17-74.21)	38.31 $\pm$ 6.52 (5.21-79.37)
	07/07/2008	6.75 $\pm$ 1.40 (0.53-12.74)	21.13 $\pm$ 3.95 (2.13-39.15)	27.87 $\pm$ 4.97 (2.66-51.89)
Banner County 5 (Wheat-Fallow)	05/23/2008	0.64 $\pm$ 0.13 (0.24-1.60)	1.25 $\pm$ 0.21 (0.26-2.27)	1.90 $\pm$ 0.27 (0.52-3.53)
	06/10/2008	1.06 $\pm$ 0.48 (0-5.00)	3.95 $\pm$ 0.84 (0.97-10.00)	5.01 $\pm$ 2.09 (1.46-11.43)
	06/23/2008	0.91 $\pm$ 0.19 (0-1.79)	25.52 $\pm$ 5.77 (10.16-68.63)	26.43 $\pm$ 5.81 (10.16-69.61)
	07/07/2008	6.27 $\pm$ 1.83 (0.80-16.00)	20.68 $\pm$ 5.75 (3.72-68.00)	26.95 $\pm$ 7.29 (4.52-84.00)

## (Appendix F. Continued)

Field (Crop Rotation)	Date	Mean $\pm$ SE (range)		
		Thrips Adults	Thrips Larvae	Total Thrips
Cheyenne County 4 (Wheat-Millet- Fallow-Wheat- Summer Crop- Fallow)	05/21/2008	3.13 $\pm$ 1.10 (0-11.98)	11.95 $\pm$ 1.70 (4.90-20.00)	15.08 $\pm$ 1.89 (6.37-25.00)
	06/09/2008	5.85 $\pm$ 1.01 (0.94-11.21)	31.27 $\pm$ 5.09 (13.21-68.37)	37.12 $\pm$ 5.24 (14.15-73.98)
	06/24/2008	3.08 $\pm$ 0.86 (0.44-7.61)	46.44 $\pm$ 9.86 (10.09-113.89)	49.52 $\pm$ 10.51 (10.53-120.83)
	07/08/2008	10.52 $\pm$ 1.85 (3.21-19.79)	24.21 $\pm$ 5.51 (4.17-62.00)	34.73 $\pm$ 6.99 (9.62-80.00)
Cheyenne County 5 (Wheat-Fallow)	05/21/2008	2.13 $\pm$ 0.48 (0.42-4.76)	8.39 $\pm$ 1.61 (2.54-16.49)	10.52 $\pm$ 1.82 (2.97-19.64)
	06/09/2008	3.63 $\pm$ 0.34 (1.39-5.50)	18.77 $\pm$ 3.53 (7.99-40.83)	22.40 $\pm$ 3.63 (9.38-44.58)
	06/24/2008	1.67 $\pm$ 0.57 (0-5.00)	46.37 $\pm$ 10.29 (25.00-134.30)	48.04 $\pm$ 10.27 (26.09-134.30)
	07/08/2008	11.90 $\pm$ 1.54 (4.52-20.65)	18.60 $\pm$ 2.06 (7.95-28.41)	30.50 $\pm$ 3.36 (14.77-44.32)
Cheyenne County 6 (Wheat-Corn- Fallow)	05/21/2008	2.59 $\pm$ 0.58 (0.43-4.67)	7.20 $\pm$ 2.10 (1.99-22.88)	9.79 $\pm$ 2.53 (2.56-27.54)
	06/09/2008	2.93 $\pm$ 0.79 (0.72-8.70)	8.77 $\pm$ 2.22 (1.65-26.63)	11.70 $\pm$ 2.90 (3.30-35.33)
	06/24/2008	0.96 $\pm$ 0.25 (0-2.63)	58.09 $\pm$ 11.74 (12.65-117.73)	59.04 $\pm$ 11.86 (12.94-119.09)
	07/08/2008	5.59 $\pm$ 1.26 (1.14-13.60)	15.11 $\pm$ 2.76 (5.26-30.70)	20.69 $\pm$ 3.86 (9.21-44.30)

Appendix G: Mean  $\pm$  standard error (SE) number of thrips adults, larvae, and total thrips per 25 sweep samples in 2008.

Field (Crop Rotation)	Date	Mean $\pm$ SE (range)		
		Thrips Adults	Thrips Larvae	Total Thrips
Banner County 3 (Wheat-Fallow)	06/10/2008	0	0.25 $\pm$ 0.25 (0-1)	0.25 $\pm$ 0.25 (0-1)
	06/23/2008	6.75 $\pm$ 1.18 (5-10)	2.00 $\pm$ 0.71 (0-3)	8.75 $\pm$ 1.65 (5-13)
	07/07/2008	4.00 $\pm$ 1.47 (1-8)	6.5 $\pm$ 1.66 (2-9)	10.5 $\pm$ 2.99 (3-17)
Banner County 4 (Wheat-Fallow)	06/10/2008	0.25 $\pm$ 0.25 (0-1)	0.50 $\pm$ 0.59 (0-1)	0.75 $\pm$ 0.25 (0-1)
	06/23/2008	3.25 $\pm$ 2.25 (1-10)	16.25 $\pm$ 0.37 (5-24)	19.50 $\pm$ 5.24 (58-91)
	07/07/2008	1.00 $\pm$ 0.36 (0-2)	1.50 $\pm$ 0.0.29 (1-2)	2.50 $\pm$ 0.29 (2-3)
Banner County 5 (Wheat-Fallow)	06/10/2008	0.75 $\pm$ 0.48 (0-2)	3.00 $\pm$ 2.04 (0-9)	3.75 $\pm$ 2.46 (0-11)
	06/23/2008	8.75 $\pm$ 2.25 (5-15)	65.00 $\pm$ 5.05 (53-76)	73.75 $\pm$ 6.86 (58-91)
	07/07/2008	1.50 $\pm$ 1.19 (0-5)	6.50 $\pm$ 2.40 (2-12)	8.00 $\pm$ 2.92 (3-14)

## (Appendix G. Continued)

Field (Crop Rotation)	Date	Thrips Adults	Mean ± SE (range)	
			Thrips Larvae	Total Thrips
Cheyenne County 4 (Wheat-Millet- Fallow-Wheat- Summer Crop- Fallow)	05/21/2008	28.25 ± 11.31 (1-51)	25.25 ± 11.77 (4-56)	53.50 ± 20.87 (5-107)
	06/09/2008	3.75 ± .48 (3-5)	7.00 ± 0.82 (5-9)	11.00 ± 1.00 (8-12)
	06/24/2008	3.00 ± 0.91 (1-5)	13.75 ± 2.14 (11-20)	16.75 ± 1.65 (13-21)
	07/08/2008	0.50 ± 0.29 (0-1)	0.50 ± 0.29 (0-1)	1.00 ± 0.58 (0-2)
Cheyenne County 5 (Wheat-Fallow)	05/21/2008	6.75 ± 3.54 (2-17)	2.25 ± 1.31 (0-6)	9.00 ± 4.71 (3-23)
	06/09/2008	5.25 ± 1.65 (1-9)	14.75 ± 6.43 (3-31)	20.00 ± 7.83 (4-40)
	06/24/2008	2.50 ± 1.85 (0-8)	6.25 ± 2.56 (1-13)	8.75 ± 4.27 (2-21)
	07/08/2008	0.75 ± 0.48 (0-2)	4.00 ± 1.58 (0-7)	4.75 ± 1.65 (0-7)
Cheyenne County 6 (Wheat-Corn- Fallow)	05/21/2008	3.50 ± 1.26 (0-6)	3.25 ± 1.31 (1-6)	6.75 ± 2.39 (1-12)
	06/09/2008	24.00 ± 5.43 (16-39)	34.75 ± 9.54 (15-60)	58.75 ± 13.70 (40-99)
	06/24/2008	2.00 ± 0.71 (1-4)	7.75 ± 1.44 (6-12)	9.75 ± 2.14 (7-16)
	07/08/2008	0	1.75 ± 0.75 (1-4)	1.75 ± 0.75 (1-4)