# ASSESSING CEREAL APHID DIVERSITY AND BARLEY YELLOW DWARF RISK IN

## HARD RED SPRING WHEAT AND DURUM

A Thesis Submitted to the Graduate Faculty of the North Dakota State University of Agriculture and Applied Science

By

Samuel Arthur McGrath Haugen

# In Partial Fulfillment of the Requirements for the Degree of MASTER OF SCIENCE

Major Department: Plant Pathology

March 2018

Fargo, North Dakota

# North Dakota State University **Graduate School**

#### Title

## Assessing Cereal Aphid Diversity and Barley Yellow Dwarf Risk in Hard Red Spring Wheat and Durum

## By

Samuel Arthur McGrath Haugen

The Supervisory Committee certifies that this disquisition complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

#### MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Andrew Friskop

Co-Chair

Dr. Janet Knodel

Co-Chair

Dr. Zhaohui Liu

Dr. Marisol Berti

Approved:

4/10/18 Date

Dr. Jack Rasmussen Department Chair

#### ABSTRACT

Barley yellow dwarf (BYD), caused by *Barley yellow dwarf virus* and *Cereal yellow dwarf virus*, and is a yield limiting disease of small grains. A research study was initiated in 2015 to identify the implications of BYD on small grain crops of North Dakota. A survey of 187 small grain fields was conducted in 2015 and 2016 to assess cereal aphid diversity; cereal aphids identified included, *Rhopalosiphum padi*, *Schizaphis graminum*, and *Sitobion avenae*. A second survey observed and documented field absence or occurrence of cereal aphids and their incidence. Results indicated prevalence and incidence differed among respective growth stages and a higher presence of cereal aphids throughout the Northwest part of North Dakota than previously thought. Field and greenhouse screenings were conducted to identify hard red spring wheat and durum responses to BYD. Infested treatments in the greenhouse had significantly lower number of spikes, dry shoot mass and yield.

#### ACKNOWLEDGEMENTS

The continuation of my education and success of this research could not have been remotely possible without the guidance, support, and expertise of many people. A very special thank-you to my advisors, Dr. Andrew Friskop and Dr. Janet Knodel. Dr. Friskop was a great mentor that always opened his door for me with enthusiasm and support every time I had a question or needed any assistance. Dr. Knodel provided a great deal of entomology guidance that broadened my scope of the project. I would also like to thank my other committee members: Dr. Zhaohui Liu and Dr. Marisol Berti for providing excellent guidance and support over the project and during my Master's education.

The generous financial support of the North Dakota Wheat Commission is greatly appreciated. I would like to thank Dr. Jack Rasmussen, Chair of the Department of Plant Pathology, for the phone call that made me excited and eager to visit NDSU and view the excellent Plant Pathology program at NDSU. I would also like to thank the NDSU Extension Service for allowing and supporting me to complete my education.

I always looked forward to the late night and some early mornings because I was able to work with absolute best crew anyone could ask for. Casey Schuh, Bryn Halley, Elizabeth Crane, Jessica Halvorson, Dr. Ryan Humann, Brandt Berghuis, Jaime Lundquist, Bryan Hanson, Christian Steffen, Jacob Lardy, Jarrett Lardy, Scott Meyer, Matt Breiland, and Jim Jordahl provided many hours of hard work, guidance, great laughs, and everlasting friendships. Thankyou to all my friends from Fargo and University of Minnesota Crookston for pushing me to go for it. Thank you again for your support.

My Mother and Father taught me the value of hard work and how to enjoy life. They spent many hours working me and listening about my graduate experience. I am forever thankful

iv

for their love and support. Thank you to my sister and brother-in-law, for being there whenever we needed extra hands and needed laughs. Finally, I would like to thank the Good Lord above for watching down every step of the way.

ABSTRACT	iii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
LITERATURE REVIEW	1
History and Diversity of Small Grain Crops	1
History of Barley yellow dwarf virus	3
Barley Yellow Dwarf Symptoms	4
Barley yellow dwarf virus and Cereal yellow dwarf virus	4
Vectors of BYDV - Cereal Aphids	6
Management	8
Literature Cited	11
CHAPTER ONE: SURVEY OF BARLEY YELLOW DWARF VECTORING CEREAL APHIDS IN SMALL GRAIN FIELDS OF NORTH DAKOTA	15
Introduction	15
Materials and Methods	17
Results	19
Discussion	31
Literature Cited	32
CHAPTER TWO: ASSESSMENT OF YIELD PARAMETERS OF HARD RED SPRING WHEAT AND DURUM WHEAT WHEN INFECTED WITH BYDV	34
Introduction	34
Materials and Methods	35
Results	38
Discussion	41

# **TABLE OF CONTENTS**

ed
ed4

# LIST OF TABLES

Table	<u>e</u>	<u>Page</u>
1.1.	Number of fields surveyed for each market class in 2015 and 2016	20
1.2.	Prevalence of cereal aphids across four market classes in 2015 and 2016 along with corresponding statistics	21
1.3.	Prevalence of cereal aphids across six growing regions in 2015 and 2016 along with corresponding statistics	22
1.4.	Prevalence of cereal aphids across three growth stages in 2015 and 2016 along with corresponding statistics	22
1.5.	Prevalence of aphid species with respect to small grain growth stage in 2015	27
1.6.	Prevalence of aphid species with respect to small grain growth stage in 2016	29
2.1.	Least squares means comparing the effect of infestation on number of spikes, dry shoot mass, and yield for hard red spring wheat	40
2.2.	Least squares means comparing the effect of infestation on number of spikes, dry shoot mass, and yield for durum	40
2.3.	Least squares means reflecting effect size differences among hard red spring wheat varieties for number of spikes, dry shoot mass, and yield for hard red spring wheat	40
2.4.	Least squares means reflecting effect size differences among hard red spring wheat varieties for number of spikes, dry shoot mass, and yield for durum	40

# LIST OF FIGURES

<u>Figur</u>	<u>e</u>	Page
1.1.	State of North Dakota map depicting the six growing regions that were used to sort aphid field prevalence data	19
1.2.	Location and prevalence of cereal aphids in 1098 small grain fields of North Dakota, in 2015.	20
1.3.	Location and prevalence of cereal aphids in 932 small grain fields of North Dakota in 2016.	21
1.4.	Cereal aphid prevalence at the Z 10-29 growth stages of small grain fields in North Dakota, in 2015.	23
1.5.	Cereal aphid prevalence at the Z 10-29 growth stages of small grain fields in North Dakota, in 2016	23
1.6.	Cereal aphid prevalence at the Z 30-79 growth stages of small grain fields in North Dakota, in 2015.	24
1.7.	Cereal aphid prevalence at the Z 30-79 growth stages of small grain fields in North Dakota, in 2016.	24
1.8.	Cereal aphid prevalence at the Z 80-96 growth stages of small grain fields in North Dakota, in 2015.	25
1.9.	Cereal aphid prevalence at the Z 80-96 growth stages of small grain fields in North Dakota, in 2016.	25
1.10.	Location and species designation of small grain fields surveyed for aphid diversity in North Dakota, in 2015	26
1.11.	Sitobion avenae prevalence among surveyed fields in 2015.	27
1.12.	Rhopalosiphum padi prevalence among surveyed fields in 2015	28
1.13.	Schizaphis graminum prevalence among surveyed fields in 2015	28
1.14.	Location and market class designation of small grain fields surveyed for aphid diversity in North Dakota in 2016	29
1.15.	Sitobion avenae prevalence among surveyed fields in 2016.	30
1.16.	Rhopalosiphum padi prevalence among surveyed fields in 2016	30

#### LITERATURE REVIEW

Barley yellow dwarf (BYD), caused by *Barley yellow dwarf virus* (BYDV), is one of the most widespread and damaging viral diseases of grasses and small grain crops. Barley yellow dwarf can cause significant yield and quality loss to wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.) (D'Arcy & Burnett, 1995). Small grain crop production accounted for over \$1.75 billion of sales in North Dakota in 2016 (NASS, 2016). Due to the value and importance of small grains, research focusing on this yield-limiting plant disease is important.

#### **History and Diversity of Small Grain Crops**

The *Poaceae* (grass) family is a large part of the earth's plant ecosystem and a staple food source for humans and animals. Over 12,000 species of *Poaceae* have been identified within 780 genera (Christenhusz & Byng, 2016) and cover approximately 20% of the earth's surface (Shantz, 1954). The *Poaceae* family is very diverse and includes many important small grain crops that are economically important to the United States (U.S.) and many other countries.

A majority of the U.S. wheat production is grown in the Great Plains. Other areas of production include states along the East Coast, Pacific Northwest, and other small pockets. Wheat cultivars are classified by the season that they are planted, such as spring wheat or winter wheat. Within the two classifications of wheat, there are six major market classes grown in the U.S. and they differ in their end uses.

Hard red winter wheat (HRWW) is the most predominately grown wheat in the U.S. typically accounting for 40 percent of the total production. Hard red winter wheat is grown throughout the Great Plains and is processed for yeast breads and hard rolls (USDA, 2016). Over the last eight years, Kansas has been the leading producer of HRWW (Plains Grain, 2016).

Soft red winter wheat (SRW) is grown mainly east of the Mississippi River and accounts for approximately 10 to 15 percent of the total U.S wheat production. Soft red wheat is primarily produced for the cracker and pastry industries (U.S. Wheat, 1990).

Spring durum (*Triticum durum* L.) is grown in the north central region of the U.S. accounting for 3 to5 percent of the total U.S. wheat production annually (USDA, 2016). North Dakota and Montana are the leading states for durum production followed by Arizona and southern California. Durum is mainly grown for semolina middlings, which is used to create pasta (U.S. Wheat, 1990).

Hard red spring wheat (HRSW) is the highest protein wheat of the six market classes and is used for hard rolls, yeast breads, and often blended with lower protein wheat (Wheat Associates, 1990). Hard red spring wheat accounts for approximately 20 percent of the U.S. total wheat production (USDA, 2016). The northern part of the Great Plains is the leader for HRSW with North Dakota leading total U.S. production.

Hard white wheat (HWW) and soft white wheat (SWW) are grown throughout parts of California and the Pacific Northwest. White wheat accounts for 10 to 15 percent of the total U.S. wheat production (USDA, 2016). Hard white wheat is grown for hard rolls, and yeast breads and SWW is used for crackers, noodles, pastries, and cakes (U.S. Wheat, 1990).

Production of barley in the U.S. takes place predominately west of the Mississippi River with Idaho, Montana, and North Dakota as the top three producing states (NASS, 2016). As with wheat, barley also has spring and winter types. Spring barley is used in the malting industry, while winter barley is used in commonly used as cover crop and livestock feed.

Two types of barley, two-row and six-row, are cultivated in the U.S. Six-row barley is used for large scale malting and brewing and livestock feed. Two-rowed barley has lower protein and higher starch content making it more desirable for micro-brewers and specialty brewers (Ullrich, 2011).

Oats are grown throughout the U.S. for milling and livestock feed (Kulp & Ponte, 2000). Wisconsin, Minnesota, and North Dakota have been the leaders of production in recent years (NASS, 2016). Oat production in the U.S. has been declining since the 1950's due to lack of market interest.

#### History of Barley yellow dwarf virus

Originally barley yellow dwarf (BYD) was commonly misidentified as various crop stresses, such as nitrogen deficiency, water stress, but never correlated as a disease. In 1951, BYD was first described by Oswald and Houston in barley plants at the University of California-Davis plant pathology research fields (Oswald & Houston, 1951). The research fields had above average number of cereal aphids present, feeding on barley during the growing season, this resulted in severe stunting and brilliant yellowing of the plants. Yield loss incurred at harvest within fields that displayed stunting and yellowing plant symptoms. Throughout the same year, commercial growers throughout the Sacramento Valley also experienced and observed the same effects in their commercial barley fields with significant yield losses. Researchers recognized the correlation between aphid-feeding and symptom occurrence in affected fields, therefore prompting greenhouse experiments to replicate field observations. Researchers were able to create the exact symptoms by rearing cereal aphids collected from the infected fields and having them feed on healthy barley plants grown in the greenhouse. Yield loss and inhibiting plant growth occurred.

Continued research took place in 1953 observing the effect of BYD on host range, planting date, and varietal reaction to further understand BYD (Oswald and Houston, 1953).

Results found that wheat, oat, and barley were hosts of the virus. Early growth stage inoculation of cereal aphids was more conducive of decreasing yield potential on all three crops. Infection of BYD could only occur when infected cereal aphids were feeding. Three cereal aphid species were tested and confirmed as vectors of the virus.

#### **Barley Yellow Dwarf Symptoms**

Symptoms of BYD include stunting, chlorosis, and yellowing of leaf tips extending towards the plant stem. Purpling and reddening of leaves may also occur and is obvious on infected oat plants. Plants with BYD are often found in pockets of the field coinciding with aphid feeding and movement. Although obvious symptoms of BYD can be observed, infected plants may also be asymptomatic and incur yield losses. Field diagnosis can be difficult as many abiotic stresses and nutrient deficiencies, such as leaf tip yellowing and burning can mimic BYD symptoms (D'Arcy & Burnett, 1995).

#### Barley yellow dwarf virus and Cereal yellow dwarf virus

Barley yellow dwarf is caused by *Barley yellow dwarf virus* and *Cereal yellow dwarf virus*. Both viruses are phloem limited, positive sense single stranded RNA viruses belonging to Luteoviridae family. The size of the virus is 25-28 nm in diameter with an icosahedral shape. *Barley yellow dwarf virus* is a luteovirus and has multiple strains that are found throughout cereal growing regions of the world such as; BYDV-PAV, BYDV-MAV, BYDV-SGV, and others. (D'Arcy & Burnett, 1995). *Cereal yellow dwarf virus* is a Polerovirus and the most common strain is CYDV-RPV (formerly BYDV-RPV). In North Dakota, the most predominant strains are BYDV-PAV and BYDV-MAV (Burrows et al., 2009). The most common CYDV strain is CYDV-RPV. Members of the Luteoviridae tend to have RNA genomes ranging in 5.5-6 kb in size with BYDV-PAV having a genome size of 5,677 nucleotides (Miller et al., 1988). Six

open reading frames (ORF) have been identified for BYDV. Each ORF has been linked to a functional use of BYDV: ORF1 and ORF2 have been linked as an essential requirement for virus replication in oats (Mohan et al., 1995); ORF3 encodes a coat protein that is needed for accumulating genomic RNA (Mohan et al., 1995); ORF4 is needed for systemic spread in a plant (Chay et al., 1996); ORF5 enocdes for a protein and is used as a read-through domain (Cheng, et al.); and the sequence flanking ORF6 is required for PAV replication in oats (Mohan et al., 1995).

Damage and disruption to host tissue occurs when the virus is able to be injected into plant cells and the virus is able to start replicating in the infected cell(s). The virus is able to spread from cell to cell using plasmodesmata and is facilitated by a movement protein (Choudhury et al., 2017). The virus causes necrotic destruction of the cell, creating phloem degeneration within the leaf. Sieve elements, nearby parenchyma cells, and companion cells in both the small vascular bundles of the leaf are affected first within the plant (D'Arcy & Burnett, 1995). This leads to a restriction of photosynthate and carbohydrate translocation to host tissue. As carbohydrate accumulation in leaves increases, inhibition of photosynthesis occurs increasing respiration rate (Jensen, 1968). Infection of roots result also occurs with degradation of pericylic and phloem parenchyma cells being the structures that are most affected. The alteration in root tissue leads to a reduction in the translocation of essential nutrients (Choudhury et al., 2017).

To confirm the presence of BYDV, enzyme linked immunosorbent assay (ELISA) or real time polymerase chain reaction (RT-PCR) are often used to confirm the presence of the virus. *Barley yellow dwarf virus* strains are routinely differentiated using serological properties (Robertson et al., 1991). Real-time polymerase chain reaction techniques have also been

developed for detection in single aphids (Canning et al., 1996; Fabre et al., 2003), which could be used in future aphid sampling studies in the Northern Great Plains.

## **Vectors of BYDV - Cereal Aphids**

Cereal aphids (Insecta: Hemiptera: Aphididae) are the major vectors of BYDV. Over 25 different species of cereal aphids can transmit BYDV and/or CYDV (D'Acry and Burnett, 1995). In North Dakota, cereal aphid migrate annually into North Dakota from overwintering populations in the southern states. Three species of cereal aphids are commonly found in small grains of North Dakota and include: bird cherry oat aphid (*Rhopalosiphum padi* (L.), English grain aphid (*Sitobion avenae* (F.), and greenbug aphid (*Schizaphis graminum* (Rondani)).

Cereal aphids can be identified by exterior characteristics, such as color, cornicles (tailpipes that protrude near the posterior end of abdomen) and venation of forewing. In general, cereal aphids have a pear-shaped body and adults can have wings (alate form) or no wings depending on its physiological state and host suitability (Dixon, 1971; Dixon, 1976; Way & Banks, 1967). Immature aphids (or nymphs) are smaller than adults and without wings. *Rhopalosiphum padi* is an oval, dark olive green to black aphid with a distinct reddish-brown patch across the lower abdomen (Blackman & Eastop, 1984). *Sitobion avaenae* is the larger of the two other cereal aphids in North Dakota. This aphid is a bright green with black cornicles and long black antennae (Blackman & Eastop, 1984). *Schizaphis graminum* is a pale yellow-green with a dark green stripe in the middle of the back (Blackman & Eastop, 1984). The median vein of the front wings is two-forked for *R. padi* and *S. avaenae*, while *S. graminum* has only one-forked median vein.

Ingwell et al. (2012) observed that plant viruses can directly change insect behavior to increase the spread of the virus; for example, *R. padi* infected with BYDV preferred to feed on

noninfected wheat plants; in contrast, noninfected *R. padi* preferred to feed on BYDV-infected plants. Ingwell et al. (2012) called this "vector manipulation hypotheses" to explain how plant pathogens affect their vectors behavior to enhance virus spread to new hosts. Other studies have shown that vectors feeding on virus-infested host plant exhibit faster growth rates, higher fecundity, increased longevity, and/or enhanced production of alate forms of the vector, which leads to increased virus spread (Gilow, 1980; Ajayi and Dewar, 1983; Araya and Foster, 19897; Ferreres and Moreno, 2009).

Cereal aphids feed on the phloem of plant tissue (stem or leaf) using needle-like piercingsucking mouthparts (Miller & Rasochova, 1997). When feeding on phloem from BYDV infected plants, cereal aphids will acquire the virus into its foregut, the virions will move in a nonpropagative, persistent, circulative manner moving from the foregut, throughout the body, and eventually reaching the salivary glands. Once the viruliferous aphid moves to a new plant, it will first probe the plant tissue to determine if it is a host and in the process transmit the virions. At least 30 min of feeding is required for the aphid to acquire the virus and will remain in the aphid for 3 to 4 day for optimum transmission. (Gray and Gildow, 2003).

Cereal aphids have evolved vector-specificity for CYDV and BYDV strains (Gray and Gildow, 2003). Different virus strains determined the species of aphid that acquires and transmits a particular virus strain (Bruehl, 1958; Rochow, 1958). As a result, each virus is named by the acronym of its most efficient aphid vector:

- BYDV-MAV is most efficiently vectored by *Macrosiphum (Sitobion) avenae*.
- CYDV-RPV is efficiently vectored by *Rhopalosiphum padi* and to a lesser extent by *S*. *graminum*.

• BYDV-PAV is vectored non-selectively with vectors such as *R. padi*, *S. avenae*, and *S. graminum* (Gray et al., 1991).

Cereal aphids reproduce actively at 20 to 27 °C (Dixon, 1971) and move by wind currents, therefore quickly moving into the northern Great Plains. Cereal aphids have not been recorded to overwinter in North Dakota; however, cereal aphids have been arriving earlier than normal since 2013 (Knodel, 2013).

#### Management

Over 150 grass species are hosts to BYDV and CYDV, including wheat, oat, barley, and rye (*Secale cereale* L.). Significant economic loss from BYD can occur with yield losses ranging from 10% to complete crop loss (D'Arcy & Burnett, 1995), therefore management is crucial to help avoid deleterious effects of the disease.

Planting date is an effective management tool used by producers to help limit BYD risk (Bockus et al., 2015). Seeding early in the season will allow plants to be at a later growth stage when infected by BYDV/CYDV, thus decreasing the amount of time for aphid feeding and virus replication (Comeau, 1987). Cereal grains infected with BYDV at early growth stages can experience greater yield and economic loss. A study conducted by Riedell et al. from 1975 to 1979 observed the effect of natural infection of BYDV on early- and late-planted oat, barley, and wheat and found a yield losses up to 46% due to BYD infection.

Seeding rates influence aphid survival throughout the growing season. Higher seeding rates will decrease the amount of air flow, providing a suitable environment for aphid feeding and reproduction (Knodel personal communication).

Screening for tolerant BYDV cultivars has predominantly been researched in oat, winter wheat, and barley cultivars in the Great Plains and southern parts of the U.S. Screenings are

intensive projects for researchers because of the interactions between cereal aphids and viruses. Identifying exact resistance genes toward BYDV can be difficult with the multiple strains of the disease occurring in nature. Coat protein-mediated resistance toward BYDV has been identified on oat when inoculated with BYDV-PAV, BYDV-MAV, and CYDV-RPV (McGrath et al., 1997). However, there is few reports on the level of tolerance of North Dakota hard red spring wheat and durum cultivars to BYD.

Barley cultivars in North Dakota were screened in 1989 and 1990 to observe effect on grain yield and impact on malting quality. Three commonly grown North Dakota malting barley cultivars were planted in the field and inoculated with greenhouse-reared viruliferous (BYDV-PAV) cereal aphids. Grain yield of all cultivars inoculated with BYDV were significantly reduced when compared with the non-inoculated plants. However, protein content was increased from 4.6% to 17.5% when plots were inoculated. A reduction of photosynthesis and sucrose translocation occurs in BYDV- infected barley, therefore increasing the accumulation of sugars and starch in the leaves and giving energy to increase protein values of the barley seed (Edwards et al., 2000).

In 1982 and 1984, Purdue University conducted field trials to observe BYDV-RPV (CYDV-RPV), and BYDV-PAV effect on oat, barley, and wheat. Data revealed that both strains of the disease caused reduction of plant height, number of tillers per plant, and grain yield. Baltenberger et al. (1987) compared inoculation of both BYDV-RPV and BYDV-PAV strains to a single strain inoculation of BYDV on wheat.

South Dakota researchers conducted experiments on the impact of physiological factors of BYDV to three different cultivars of HRSW (Jensen and Van Sambeek, 1971). Researchers found that all three cultivars had varying degrees of yield and photosynthetic rate reduction. A

72% loss of photosynthetic capacity of the flag leaf was observed in the wheat cultivars tested. Researchers concluded that when susceptible plants are infected with BYDV the culm and spike are the main areas of photosynthetic activity, which promotes yield.

Cultivar tolerance toward BYDV in spring wheat and durum was studied in 1964 by researchers in southern Manitoba (Gill, 1967). The study included nine cultivars of common spring wheat as well as four durum cultivars. The study took place in greenhouse and field environments where viruliferous cereal aphids fed on the plants and transferred BYDV. Yield losses were observed on both the field and greenhouse trials. Plants in the greenhouse trial had more classic symptoms of BYD than those in the field trials, with plants showing heavy yellowing and chlorosis. In the field studies, however, major stunting of the plants, but minimal yellowing and chlorosis was observed. The researchers concluded the shortage of leaf coloring symptoms in the field trials could be due to the later timing of inoculation of BYDV.

Management using chemicals can only be used to reduce populations of the aphid vector and to mitigate the spread of the virus. Field scouting for cereal aphid should begin at stem elongation and continue up to the completion of the heading stage. Producers in North Dakota are recommended to treat with insecticides when 85 percent of the stems have one or more cereal aphids or 12-15 aphids per stem (Voss et al., 1997). If aphid populations exceed the economic threshold, yield loss from aphid feeding injury may occur. To prevent yield loss, foliar insecticide sprays are recommended when cereal aphids reach the economic threshold and when the crop is still susceptible to aphid feeding injury, up to the completion of heading (Knodel et al., 2007). In the southern U.S., imidacloprid and other neonicotinoid seed treatments are a commonly used by small grain producers to help reduce levels of cereal aphids and risk of BYDV in the field. A yield increase of 112% was observed when using imidacloprid seed

treatments under a moderately susceptible cultivar that was inoculated with viruliferous cereal aphids carrying BYDV-PAV-IL (Gourmet et al., 1996).

Beneficial insects, such as lady beetles, lacewings, and parasitic wasps can be effective in reducing cereal aphid populations. Brewer and Elliot (2004) found that the parasitoid fauna was effective in managing cereal aphids on small grain crops. Research on many beneficial organisms is limited in small grains, but opportunities exist for more sustainable pest management of cereal aphids (Brewer and Elliott, 2004). At the present, biological control is not an effective tool against aphid-virus management; however, future integrated pest strategies could provide more efficient control, such as virus-resistant cultivars and conservation of natural enemies of cereal aphids.

#### **Literature Cited**

- Ajaji, O. and Dewar, A.M. 1983. The effect of *Barley yellow dwarf virus* on field populations of the cereal aphids, *Sitobion avenae* and *Metopolophium dirhodum*. Ann. Appl. Bio. 103, 1-11.
- Araya, J.E. and Foster, J.E. 1987. Laboratory study on the effects of *Barley yellow dwarf virus* on the life cycle of *Rhopalosiphum padi* (L.). J. Plant Dis. Protect. 94, 195-198.
- Blackman, R. and Eastop, V. 1984. Aphids on the World's Crops: An Identification Guide. Chichester: John Wiley and Sons. 341-351
- Bockus, W., De Wolf, E. D., and Todd, T. 2015. Effects of planting date, cultivar, and imidaclorprid seed treatment on barley yellow dwarf virus of winter wheat, 2012-2014. Plant Health Progress. 17, 122-127.
- Burrows, M., Franc, G., Rush, C., Blunt, T., Dai, I., Kinzer, K. and Stack, J. 2009. Occurrence of viruses in wheat in the Great Plains Region, 2008. Plant Health Progress. Web access only.
- Canning, E. S., Penrose, M. J., Barker, I., Coates, D. 1996. Improved detection of barley yellow dwarf virus in single aphids using RT-PCR. J Virol. Methods. 2, 191-7.
- Chay, C. A., Gunasinge. U. B., Dinesh-Kumar S. P., Miller W. A., and Gray, S. M. 1996 Aphid transmission and systemic plant infection determinants of barley yellow dwarf luteovirus-

PAV are contained in the coat protein readthrough domain and 17-kDa protein respectively. Virology. 219, 57–65.

- Cheng, S. L., Domier, L. L., and D'Arcy, C. J. 1994. Detection of the readthrough protein of barley yellow dwarf virus. Virology 202, 1003–1006.
- Choudhury, S., Hu, H., Meinke, H., Shabala, S., Westmore, G., Larkin, P., and Zhou, M. 2017 Barley yellow dwarf viruses: infection mechanisms and breeding strategies. Euphytica. 213, 168.
- Dixon, A.F.G. 1971. The life-cycle and host preferences of the bird cherry-oat aphid, *Rhopalosiphum padi* L., and their bearing on the theories of host alternation in aphids. Ann. Appl. Biol 68: 135-147.
- Dixon, A.F.G. 1976. Reproductive strategies of the alate morphs of the bird cherry-oat aphid, *Rhopalosiphum padi* L. J. Anim. Ecol. 45: 817-830.
- D'Arcy, C. J., & Burnett, P. A. 1995. Barley Yellow Dwarf: 40 Years of Progress. The American Phytopathological Society, St. Paul, MN. APS Press. 9-29, 75-76, 203
- Edwards, M. C., Fetch, T. G., Schwarz, P. B., & Steffenson, B. J. 2000. Effect of barley yellow dwarf virus infection on yield. Plant Dis. 85, 202-207.
- Fabre, F., Kervarrec, C., Mieuzet, L., Riault, G., Vialatte, A., Jacquot, E. 2003. Improvement of Barley yellow dwarf virus-PAV detection in single aphids using a fluorescent real time RT-PCR. J Virol Methods. 110, 51-60.
- Fereres, A. and Moreno, A. 2009. Behavioural aspects influencing plant virus transmission by homopteran insects. Virus Res. 141, 158-168.
- Gildow, F.E. 1980. Increased production of alate by aphids reared on oats infected with *Barley yellow dwarf virus*. Ann. Entomol. Soc. Am. 73, 343-347.
- Gourmet, C., Kolb, F., Smyth, C., and Pedersen, W. 1996. Use of imidacloprid as a seedtreatment insecticide to control barley yellow dwarf virus (BYDV) in oat and wheat. Plant Dis. 136-141.
- Gray, S., and Gildow, F. 2003. Luteovirus-aphid interactions. Am. Phytopath, 539-566.
- Gray, S., Power, A., Smith, D., Seaman, A., and Altman, N. 1991. Aphid transmission of arley yellow dwarf virus: Acquisition access periods and virus Concentration Requirements. Phytopathology, 539-545.
- Hesler, L.S. and Berg, R.K. 2003. Tillage impacts cereal-aphids (Homoptera: Aphididae) infestations in spring small grains. J. Econ. Entomol. 96, 1792-1797.

- Hull, R. 1977. Particle differences related to aphid-transmissibility of a plant virus. General Virology, 34 1983-1987.
- Hull, R. 2009. Comparative plant virology. Burlington: Elsevier Academic Press. 376 pp.

Jedlinski, H., Rochow, W. F., and Brown, C. M. 1977. Tolerance to barley yellow dwarf virus in oats. Phytopathology, 67, 1408-1411.

- Jensen, S. 1968. Photosynthesis respiration and other physiological relationships in barley infected with barley yellow dwarf virus. Phytopathology 58, 204.
- Knodel, J.J. 2013. Early Arrival of Cereal Aphids in Minnesota. NDSU Extension Service Crop and Pest Report (5). June 6, 2013.
- Knodel, J., Beauzay, P., Boetel, M., and Prochaska T. J. 2017. 2018 North Dakota Field Crop Insect Management Guide. NDSU Ext. Serv., E1143 (revised). 114 p.
- Kulp, K., and Ponte, J. J. 2000. Handbook of cereal science and technology: Second Edition. New York: Marcel Dekker. 142-144
- McGrath, P., Vincent, J., Lei, C. H., Pawlowski, W., Torbert, K., Gu, W., and Lister, R. 1997. Coat protein-mediated resistance to isolates of barley yellow dwarf in oats and barley. Eur. J. Plant Path, 103, 695–710.
- Miller, W. A., and Gerlach W. L. 1988 Sequence and organization of barley yellow dwarf virus genomic RNA. Nucleic Acids Res. 13, 97-111.
- Miller, W. A., and Rasochova, L. 1997. Barley yellow dwarf viruses. Annual Review. 35, 167-190.
- Mohan B., Dinesh-Kumar S., and Miller W. A. 1995. Genes and cis-acting sequences involved in replication of barley yellow dwarf virus-PAV RNA. Virology 212, 186–195.
- NASS. 2016. 2016 state agriculture overview-North Dakota. USDA.
- Oswald, J., and Houston, B. 1953. The yellow dwarf virus disease of cereal crops. Phytopathology, 43, 128-136.
- Oswald, J. W., and Houston, B. R. 1951. A new virus disease of cereals transmissible by aphids. Plant Disease Reporter, 11, 471-475.
- Perry, K. L., Kolb, F. L., Sammons, B., Lawson, C., Cisar, G., and Ohm, H. 2000. Yield effects of barley yellow dwarf virus in soft red winter wheat. Am. Phytopath, 9, 1043-1048.

- Robertson, N., French, R., and Gray, S. 1991. Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. J. General Virology, 1473-1477.
- Shantz, H. 1954. The place of grasslands in the earth's cover of vegetation. Ecology, 143-145.
- Ullrich, S. 2011. Barley: Production, Improvement, and Uses. Ames: Wiley-Blackwell. 4-12
- US Wheat Associates. 1990. U.S Wheat. Mandan: ND Wheat Commission.
- USDA. 2016. U.S. Wheat Classes. USDA.
- Voss, T.S., Kieckhefer, R.W., Fuller, B.W., McLeod, M.J. and Beck, D.A. 1997. Yield losses in maturing spring wheat caused by cereal aphids (Homoptera: Aphidadae) under Laboratory Conditions J. Econ. Entomol. 90, 1346-1350.
- Way, M.J. and C.J. Banks. 1967. Intra-specific mechanisms in relation to the natural regulation of numbers of *Aphis fabae* Scop. Ann. Appl. Biol. 59: 189-205.

# CHAPTER ONE: SURVEY OF BARLEY YELLOW DWARF VECTORING CEREAL APHIDS IN SMALL GRAIN FIELDS OF NORTH DAKOTA

#### Introduction

Barley yellow dwarf (BYD) is an economically important disease of cereal grains found worldwide and is caused by a complex of viruses including *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* (CYDV) (D'Arcy & Burnett, 1995). Both viruses are vectored by cereal aphids in a semi-persistent, circulative, non-propagative manner and cannot be mechanically transmitted. There are several strains of BYDV and CYDV with specific virusvector relationships. For example, BYDV-PAV can be vectored by *Rhopalosiphum padi (R. padi)*, *Schizaphis graminum (S. graminum)*, and *Sitobion avenae* (S. *avenae*) whereas CYDV-RPV is selectively transmitted by *R. padi*. In North Dakota, the most common virus causing BYD is BYDV-PAV (Burrows et al., 2009). Cereal aphids overwinter on host plants grown in the southern United States and are dependent on wind currents to move into the northern Great Plains. This often results in cereal aphids being detected during the middle of the small graingrowing season around flag leaf emergence. In 2013, an early arrival of cereal aphids in North Dakota occurred during tillering growth stages and elevated the risk for potential yield losses attributed to BYD (Knodel, 2013).

North Dakota is a major producer of spring sown small grains including hard red spring wheat (*Triticum aestivum* L.), durum (*Triticum durum* L.), and barley (*Hordeum vulgare* L.), all which are susceptible to BYD. These crops are routinely grown on over 3.2 million hectares of land each year (NASS, 2016). One of the major limiting factors of small grain production are plant diseases. Plant disease prevalence and severity can vary from year to year and can be dependent on environmental conditions and growing practices. Thus, it is important to scout and monitor pest populations every year and make decisions to help reduce economic losses.

Surveying for cereal aphids utilizes several different methodologies. One way is to scout for cereal aphids in a field and to examine the number of aphid-infested stems throughout the field. To protect small grains from grain yield loss due to aphid feeding, the economic threshold is 85% stems with more than one aphid present prior to complete heading (Knodel et al., 2017). For species diversity, the survey method used was a sweep net technique. This technique is unconventional and not widely used for cereal aphids and is more appropriate for other fastmoving insects, such as grasshoppers and leafhoppers (Knodel et al., 2017). However, due to the sporadic distribution of cereal aphids in a field, this technique was chosen to cover a greater sampling area and potentially collect more cereal aphids outside of the standard five stopping points of a field. Another aphid sampling technique that is commonly used is wind aphid suction traps (Alison & Pike, 1988). However, this method also may capture beneficial insects and skew aphid diversity readings since trap height and placement can affect aphid species collected.

Survey efforts documenting pests in North Dakota have been robust. The North Dakota Integrated Pest (IPM) Survey has been conducted by personnel at North Dakota State University for close to twenty years. This survey effort has contributed valuable information on the prevalence of diseases and insects for small grain growers in the state. Cereal aphids and BYD are two of the key pests included on the IPM Survey. The scouting technique used for cereal aphids involves presence or absence of aphids infested on the main stems from five locations throughout the field and the overall incidence of aphids is determined. Although this provides valuable information on aphid incidence, specific information on species of cereal aphids has not been documented. Understanding species diversity of cereal aphids can provide valuable

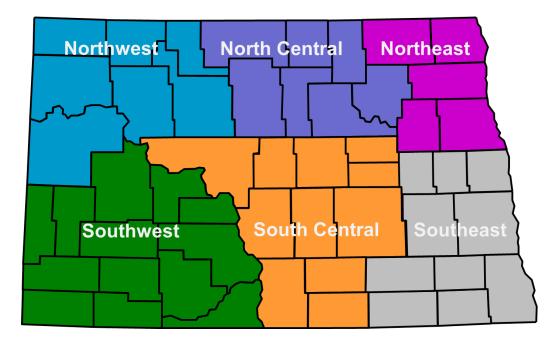
information on predicting the prevalence of BYDV and CYDV strains in North Dakota. Therefore, the objectives of this study were: (i) To report on the incidence of fields with cereal aphids in North Dakota in 2015 and 2016 and (ii) To determine species diversity in aphid samples collected from small grain fields in 2015 and 2016.

#### **Materials and Methods**

Integrated Pest Management (IPM) Survey for Cereal Aphids. Trained IPM scouts surveyed randomly selected small grain fields during 2015 and 2016. Field stops were made approximately every 24.1 km and/or dependent on the popularity of small grain fields in specific counties. Scouts recorded the GPS coordinates at each field, and walked a 'W' pattern making five stops within each field. Scouts assessed the presence or absence of cereal aphids on 20 main stems at each location in each field for a total of 100 main stem assessments. Observations of aphid pressure were used to measure aphid field prevalence. For this survey, field prevalence is defined as the percentage of fields with aphid(s) present. Field prevalence data was first sorted according to small grain market class, namely, hard red spring wheat, hard red winter wheat, spring durum, and spring barley. Data across small grain market classes was combined and sorted according to growth stage and growing region. Three Zadoks (Z) growth stage ranges were developed and included Z10-29 (seedling to tillering), Z30-79 (stem elongation to milk development) and Z80-96 (dough development to ripening) to categorize the seasonal aphid data. Six growing regions were coded as southeast, southcentral, southwest, northeast, northcentral, and northwest (Fig. 1). Growing regions were determined to align with North Dakota Wheat Commission districts. Survey maps were created using ArcGIS software (ArcGIS Server 9.3; ESRI, Redlands, CA). Using the procedure frequency in SAS 9.4 (SAS Institute, Cary, NC), a chi-square test of homogeneity was used to detect differences in aphid field prevalence across

market classes, growth stages, and growing regions. To compare differences among independent variables, least square means (lsmeans) were developed from the aphid field prevalence values. The lsmeans were than analyzed using PROC GLM with a tukey-kramer adjustment.

**Cereal Aphid Species Diversity.** Separate from IPM scouted fields, randomly selected small grain fields were scouted and assessed for cereal aphid species diversity. Field stops were made approximately 25 km apart from other field sites and a sweep net was used to collect cereal aphids. Specifically, GPS coordinates were recorded at each field site. Each field was walked in a 'W' pattern beginning 15 m into the field, and a 60.96 cm insect sweep net was used to collect cereal aphids. While walking in a ''W' pattern, 100 sweeps were conducted and contents of the sweep net were collected and stored in vials with 95% ethanol solution. Vials were emptied and aphid species were identified using a dissection microscope and insect identification resource guides (Blackman and Eastop, 1984). After identification, the number belonging to each species was counted and used to obtain aphid species prevalence. Aphid species prevalence is defined as the number of fields with a specific aphid species present. Field information and data was organized into the three growth stage ranges Z10-29, Z30-79 and Z80-96.



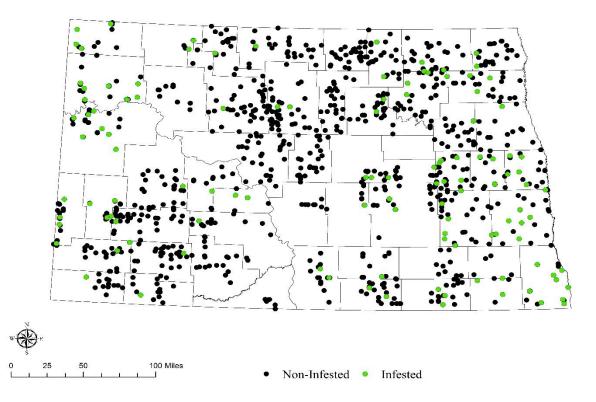
**Figure 1.1.** State of North Dakota map depicting the six growing regions that were used to sort aphid field prevalence data.

### Results

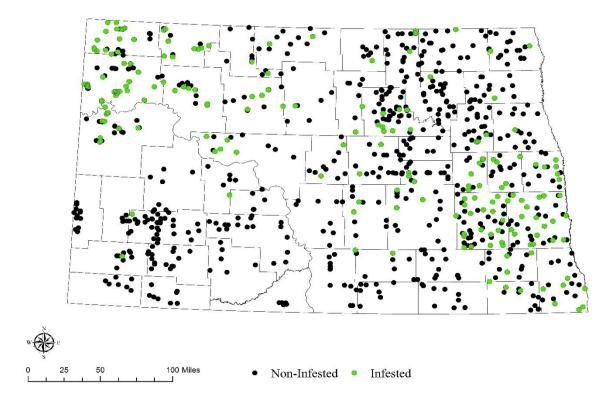
**Aphid Field Prevalence.** A total of 2,030 small grain fields were visited in 2015 and 2016 in North Dakota. In 2015, 800 hard red spring wheat, 40 winter wheat, 24 durum, and 234 barley fields were scouted (Table 1.1 and Figure 1.1). In 2016, 677 hard red spring wheat, 31 winter wheat, 109 durum and 115 barley fields were scouted (Table 1.1 and Figure 1.2). Differences in aphid field prevalence were observed pertaining to market class (Table 1.2). In both 2015 and 2016, aphid field prevalence was higher in durum when compared with all other market classes. Barley and hard red spring wheat had the lowest prevalence of cereal aphids in 2015 and 2016, respectively. Significant observations were observed when small grain data was compiled and sorted according to growing region and growth stage. In both 2015 and 2016, the growing regions of the northwest and southeast had the highest amount of small grain fields with cereal aphids (Table 1.3, Figure 1.2 and Figure 1.3). The growing regions with the lowest aphid prevalence were observed

with respect to growth stages. The fields with highest prevalence of cereal aphids occurred during Z30-79, with lower aphid prevalence occurring during Z10-29 and Z80-96 (Table 1.4, Figures 1.3 to 1.8).

Market Class	2015	2016	Total Fields Surveyed
Hard red spring wheat	800	677	1477
Winter wheat	40	31	71
Durum	24	109	133
Barley	234	115	349
Total	1098	932	2030



**Figure 1.2.** Location and prevalence of cereal aphids in 1098 small grain fields of North Dakota, in 2015.



**Figure 1.3.** Location and prevalence of cereal aphids in 932 small grain fields of North Dakota in 2016.

**Table 1.2.** Prevalence of cereal aphids across four market classes in 2015 and 2016 along with corresponding statistics.

		Market Class <sup>x</sup>				
		HRSW	HRWW	Durum	Barley	<i>P</i> -value
2015	Fields with aphids (%)	13.88	7.50	25.00	3.85	<0.0001 <sup>y</sup>
	Least square mean	1.06 a	0.68 ab	2.84 a	0.03 b	0.0062 <sup>z</sup>
2016	Fields with aphids (%)	17.43	48.39	53.21	26.09	<0.0001 <sup>y</sup>
	Least square mean	0.13 b	0.28 ab	0.28 a	0.19 ab	0.0067 <sup>z</sup>

<sup>x</sup>HRSW = Hard red spring wheat, HRWW = Hard Red Winter Wheat

<sup>y</sup>Level of significance (p-value) for chi square test of homogeneity

<sup>z</sup>Level of significance from tukey-kramer multiple comparison test. Values accompanied by different letters within columns are statistically different from each other.

Growing Regions<sup>x</sup> SW NW SC NC SE NE P value Fields with aphids (%) 8.33 14.78 7.11 5.94 29.14 10.92 <0.0001<sup>y</sup> 2015 0.03 c 0.16 b 0.08 bc 0.09 bc 0.30 a 0.12 bc <0.0001z Least square mean Fields with aphids (%) 2.10 47.12 13.99 13.57 36.32 8.25 <0.0001<sup>y</sup>

Table 1.3. Prevalence of cereal aphids across six growing regions in 2015 and 2016 along with corresponding statistics. (See Fig. 1.1 for map).

 $^{x}SW =$  southwest, NW = northwest, SC = south central, NC = north central, SE = southeast, NE =northeast

0.15 bc

0.20 b

0.13 bc

< 0.0001<sup>z</sup>

0.37 a

<sup>y</sup>Level of significance (p-value) for chi square test of homogeneity

0.03 c

2016

Least square mean

<sup>z</sup>Level of significance from tukey-kramer multiple comparison test. Values accompanied by different letters within columns are statistically different from each other.

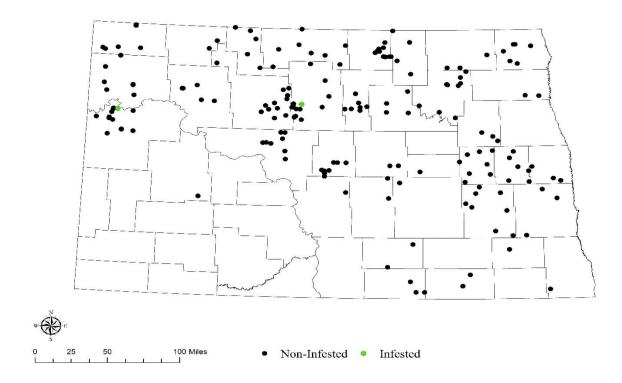
0.44 a

**Table 1.4.** Prevalence of cereal aphids across three growth stages in 2015 and 2016 along with corresponding statistics.

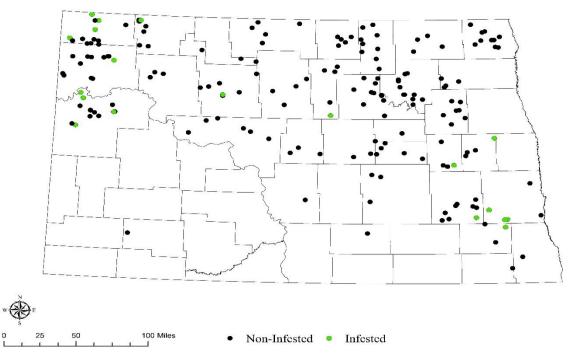
		Growth Stages			
		Z10-29	Z30-79	Z80-96	P Value
2015	Fields with aphids (%)	1.06	14.22	13.19	0.0001 <sup>y</sup>
	Least square mean	0.02 b	0.17 a	0.20 a	<0.0001 <sup>z</sup>
2016	Fields with aphids (%)	10.53	57.73	21.89	0.0001 <sup>y</sup>
	Least square mean	0.10 c	0.22 b	0.35 a	<0.0001 <sup>z</sup>

<sup>y</sup>Level of significance (p-value) for chi square test of homogeneity

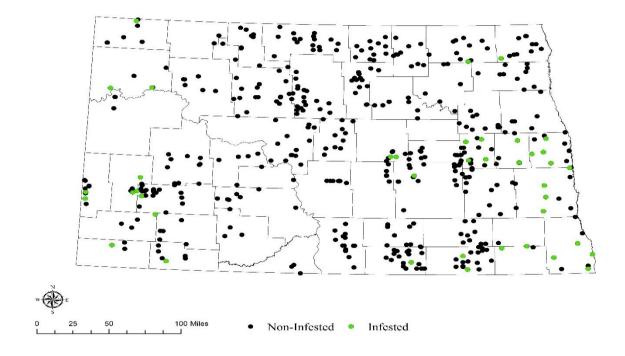
<sup>z</sup>Level of significance from tukey-kramer multiple comparison test. Values accompanied by different letters within columns are statistically different from each other.



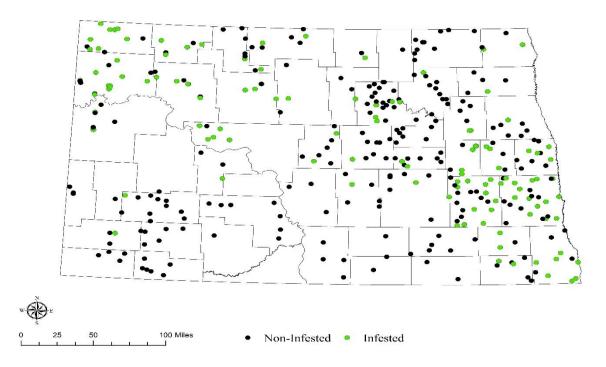
**Figure 1.4.** Cereal aphid prevalence at the Z 10-29 growth stages of small grain fields in North Dakota, in 2015.



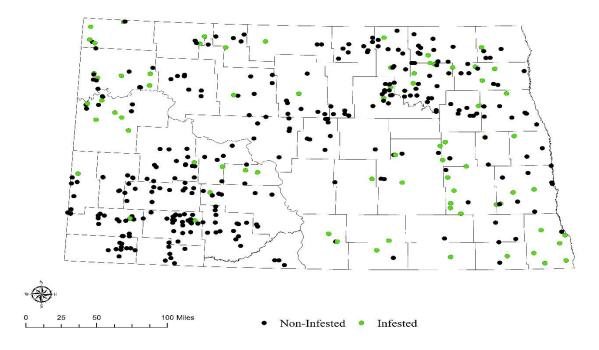
**Figure 1.5.** Cereal aphid prevalence at the Z 10-29 growth stages of small grain fields in North Dakota, in 2016.



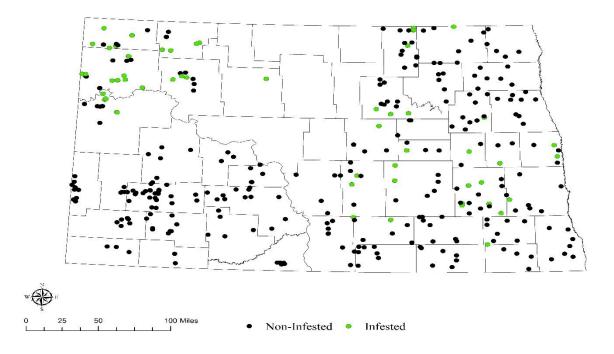
**Figure 1.6.** Cereal aphid prevalence at the Z 30-79 growth stages of small grain fields in North Dakota, in 2015.



**Figure 1.7.** Cereal aphid prevalence at the Z 30-79 growth stages of small grain fields in North Dakota, in 2016.

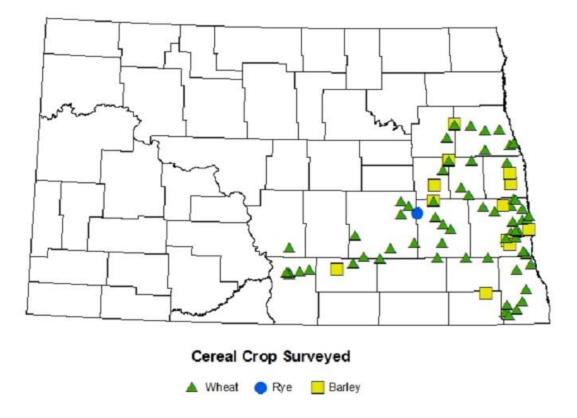


**Figure 1.8.** Cereal aphid prevalence at the Z 80-96 growth stages of small grain fields in North Dakota, in 2015.



**Figure 1.9.** Cereal aphid prevalence at the Z 80-96 growth stages of small grain fields in North Dakota, in 2016.

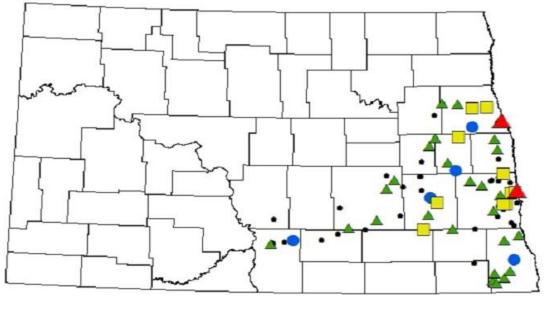
**Aphid Species Diversity.** In 2015, 80 small grain fields were surveyed for aphid species in 14 North Dakota counties. Surveyed fields included 60 hard red wheat, 12 barley, and one rye (*Secale cereale* L.) field (Figure 1.10). Three cereal aphid species were detected in 2015 included *S. avenae*, *R. padi*, and *S. graminum* (Tables 1.5; Figures 1.10 to Figures 1.13). Five surveyed fields had multiple cereal aphids species present in 2015. The most common aphid species detected in 2015 was *S. avenae* and appeared in the most fields at Z30-79 and Z80-96 (Table 1.5, Figures 1.11). In 2016, a total of 139 fields were scouted in 10 North Dakota counties including 104 hard red spring wheat, 27 barley, 2 winter wheat, and 1 durum field (Figure 1.14). Two aphid species were detected with *S. avenae* being the most commonly detected (Table 1.6; Figures 1.14 and 1.15). Seventeen surveyed fields had two cereal aphids species present in 2016. Cereal aphids were detected only during Zadoks 30-79 in 2016 (Table 1.6).



**Figure 1.10.** Location and species designation of small grain fields surveyed for aphid diversity in North Dakota, in 2015.

Number of	Approximate growth	R. padi	S. avenae	S. gramminum		
fields sampled	stage (Zadoks)	Prevalence (%)				
4	Tillering (10-29)	25.0	0	0		
47	Stem elongation-milk development (30-79)	17.0	70.2	12.7		
29	Dough development- ripening (80-96)	17.2.0	38.0	17.2		

Table 1.5. Prevalence of aphid species with respect to small grain growth stage in 2015.



Total Number of Aphids Collected per 100 Sweeps

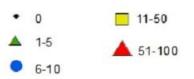


Figure 1.11. Sitobion avenae prevalence among surveyed fields in 2015.

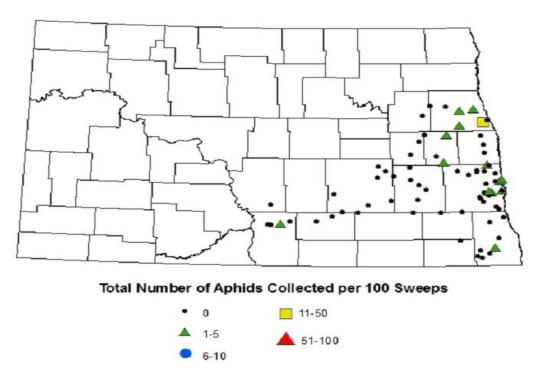


Figure 1.12. Rhopalosiphum padi prevalence among surveyed fields in 2015.

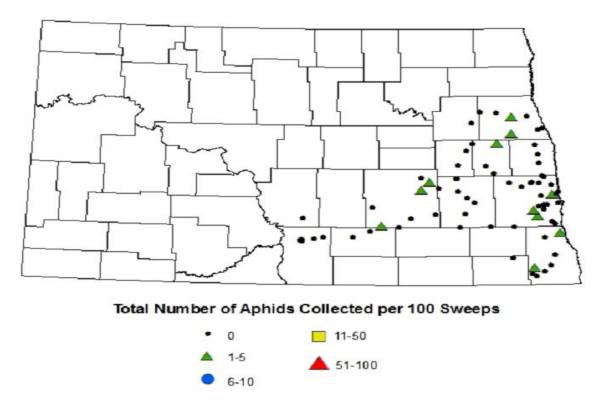
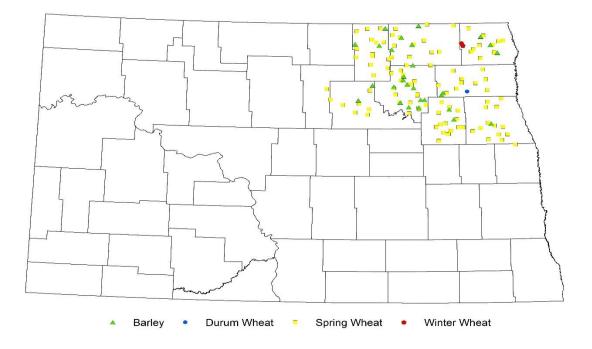


Figure 1.13. Schizaphis graminum prevalence among surveyed fields in 2015.



**Figure 1.14.** Location and market class designation of small grain fields surveyed for aphid diversity in North Dakota in 2016.

Number of	Approximate Growth	R. padi	S. avenae	S. gramminum
fields sampled	Stage (Zadoks)	Prevalence (%)		
55	Tillering (10-29)	0	0	0
32	Stem elongation-milk development (30-79)	19.5	21.5	0
52	Dough development- ripening (80-96)	0	0	0

Table 1.6. Prevalence of aphid species with respect to small grain growth stage in 2016.

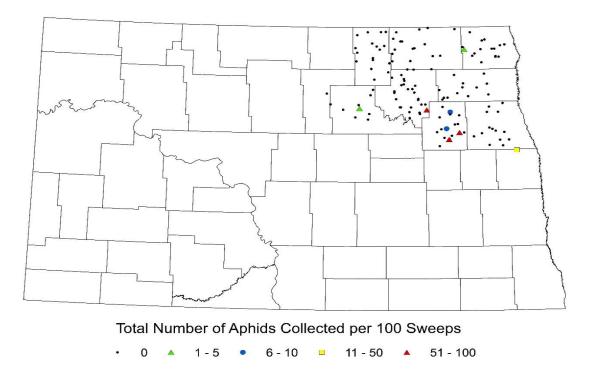


Figure 1.15. Sitobion avenae prevalence among surveyed fields in 2016.

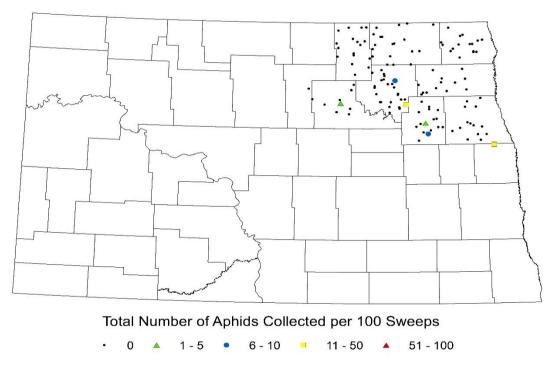


Figure 1.16. Rhopalosiphum padi prevalence among surveyed fields in 2016.

# Discussion

Prevalence of cereal aphids in small grain field has been examined for several years in North Dakota; however this is the first survey effort to document prevalence and diversity of cereal aphids across multiple growing seasons in North Dakota. The early arrival of cereal aphids has heightened awareness on the risk BYD may pose to cereals in the state. However, even though cereal aphids have arrived earlier, the highest number of fields with aphid infestation were observed after spike emergence into flowering. This may indicate that aphid infestation is often delayed during the susceptible growth stages of small grains in North Dakota. Studies that have examined economic loss have indicated the greatest losses occur when plants are infested with viruliferous cereal aphids at early leaf development stages (Bockus et al., 2015). Although sporadic high aphid infestations do develop in small grains early in the growing season, the majority of fields infested with cereal aphids occurs at later growth stages, which lowers the crop's risk for economic loss.

North Dakota is a leading state for the production of hard red spring wheat, durum, and barley. All three of these small grains routinely will harbor cereal aphids. Hard red spring wheat and barley are grown across the entire state, but most durum production is located in in northwest North Dakota. Previous yield losses attributed to BYD has been assessed to crops not tested in this study such as winter wheat, oat, and barley (Bockus et al., 2015; Perry et al. 2000; and Edwards et al., 2000). However, the impacts of BYD on durum needs further research. Given the elevated numbers of cereal aphids observed in northwestern North Dakota, a yield loss assessment study would help understand risk in this crop.

The most common cereal aphid species in small grains was *S. avenae* followed by *R. padi*. Although both aphid species serve as vectors for viruses that cause BYD, *R. padi* is the

most efficient vector for CYDV-RPV and non-selective vector for BYDV-PAV. The low incidence of this aphid also may suggest that the risk of deleterious yield losses. CYDV-RPV incidence is low in North Dakota. *Sitobion avenae* was detected more frequently than any other aphid species and it is a vector of BYDV-PAV. The prevalence of *S. avenae* supports previous virus survey work conducted on wheat and the prevalence of aphid transmitted viruses in 2008 throughout North Dakota (Burrows et al., 2008). The detection of the virus and the aphid vector may help prioritize wheat breeding efforts on BYD.

This is the first study that has documented the species diversity and prevalence of cereal aphids in North Dakota. Given the increasing risk of cereal aphids earlier arrival in the state, this study has provided some insight on potential species dynamics and distribution of cereal aphids in the state. Aphid populations were the highest towards the end of the season and the species diversity complex was driven by *S. avenae*. The combination of field prevalence, field incidence and species diversity will provide increased awareness of cereal aphid and BYD risk for the small grain crop of North Dakota.

# **Literature Cited**

- Allison, D., and Pike, K. 1988. An inexpensive suction trap and its use in an aphid monitoring network. J. Econ. Entomol., 5, 103-107.
- Blackman, R., and Eastop, V. 1984. Aphids on the World's Crops: An Identification Guide. Chichester: John Wiley and Sons. 341-351
- Bockus W., De Wolf, E. D., and Todd, T. 2015. Effects of planting date, cultivar, and imidaclorprid seed treatment on barley yellow dwarf virus of winter wheat. Plant Dis. Mgmt., 17, 122-127.
- Burrows, M., Franc, G., Rush, C., Blunt, T., Dai, I., Kinzer, K., and Stack, J. 2009. Occurrence of viruses in wheat in the Great Plains Region, 2008. Plant Health Progress. Web access only.
- D'Arcy, C. J., and Burnett, P. A. 1995. Barley Yellow Dwarf: 40 Years of Progress. St. Paul: APS Press. 9-29, 75-76, 203

- Edwards, M. C., Fetch, T. G., Schwarz, P. B., and Steffenson, B. J. 2000. Effect of barley yellow dwarf virus infection on yield. Plant Disease: 85, 202-207.
- Knodel, J. J., Beauzay, P., Boetel, M. A., and Prochaska, T. J. 2017. 2018 North Dakota Field Crop Insect Management Guide. NDSU Ext. Serv., E1143 (revised).
- NASS. 2016. 2016 State Agriculture Overview-North Dakota. USDA. Retrieved March 1, 2016 https://www.nass.usda.gov/Quick\_Stats/Ag\_Overview/stateOverview.php?state=NORTH %20DAKOTA
- Perry, K. L., Kolb, F. L., Sammons, B., Lawson, C., Cisar, G., and Ohm, H. 2000. Yield Effects of barley yellow dwarf virus in soft Red winter wheat. Am. Phytopath. Soc., 9, 1043 1048.

.

# CHAPTER TWO: ASSESSMENT OF YIELD PARAMETERS OF HARD RED SPRING WHEAT AND DURUM WHEAT WHEN INFECTED WITH BYDV

# Introduction

Barley yellow dwarf complex (BYD), caused by *Barley yellow dwarf virus* and *Cereal yellow dwarf virus*, can limit the yields of major small grains grown in North Dakota including hard red spring wheat (*Triticum asestivum* L.), barley (*Hordeum vulgare* L.), and durum (*Triticum durum* L.). Both BYDV and CYDV are vectored by cereal aphids with some having a specific vector-virus relationship (D'Arcy & Burnett, 1995). The primary symptom of BYD is chlorosis beginning at the tip of the leaf extending to the stem of the plant. The yellowing inhibits the ability of the plant perform photosynthesis leading to yield and plant loss (D'Arcy & Burnett, 1995).

There have been several studies completed on the yield loss potential of BYD in small grains. Bockus et al. (2014) observed losses up to 86% in winter wheat when inoculations occurred at early planting. This viral disease also has been linked to reductions in root and shoot growth (Perry, 2000). With regards to barley, yield loss and reduced BYD caused significant losses in yield, kernel plumpness, and thousand-kernel weight (Edwards et al., 2000). Additional findings from Edwards et al. (2000) indicated that BYDV-PAV infection increased the protein and decreased malt extract.

Management of BYD is best accomplished using an integrated strategy including cultural, chemical, and host resistance. However, in some growing systems, multiple management tools are not available or are not recommended. The major hard red winter wheat growing regions have used insecticide seed treatments to delay aphid infestations; however, insecticide seed treatments may have minimal efficacy for spring sown grains. Cereal aphids often arrive at late vegetative leaf stages (mid-June or later) in the northern Great Plains, when residual effect of seed treatment has diminished. Early planting dates are effective at avoiding peak populations of aphid feeding and reproduction, and reducing grain yield losses (Bockus et al., 2015). Host resistance is the preferred method for the management of BYD. Aphid resistance has been classified as antixenosis, antibiosis, and tolerance. Antixenosis occurs when resistance affects the behavior of an insect pest and usually is expressed as non-preference of the insect for a resistant plant compared with a susceptible plant (Painter, 1951, Smith, 1989). Antibiosis affects the biology of the insect so pest abundance and subsequent damage is reduced compared with that which would have occurred if the insect was on a susceptible crop cultivar (Painter, 1951; Radcliffe et al., 2017, Smith, 1989). Antibiosis often results in increased mortality or reduced longevity and reproduction of the insect.

Resistance to BYDV-PAV and CYDV-RPV has been explored in several wheat gene pools. *Thinopyrum* species have been shown to disrupt virus replication and a potential source of resistance (Radcliffe et al., 2017). Hard red winter wheat cultivars grown in Kansas were shown to have levels of improved resistance against barley yellow dwarf (Gaunce and Bockus, 2011).

The objective of this study was to evaluate the effects of BYD on hard red spring wheat and durum grown in North Dakota. Field studies were used to help document yield and test weight losses attributed to BYD. Greenhouse studies examined the effect of BYD on number of heads produced, dry shoot mass, and yield. Additionally, the influence of hard red spring wheat and durum cultivars on aphid reproduction were examined in the greenhouse.

#### **Materials and Methods**

**Plant Material.** Three cultivars of hard red spring wheat and two cultivars of durum were used for both the field and greenhouse experiments. Cultivars were selected based on their

respective planted acres in North Dakota during the 2015 growing season (NASS, 2105). Hard red spring wheat cultivars included WB-Mayville (WestBred), Prosper (NDSU), and Barlow (NDSU). Durum cultivars included Carpio (NDSU) and Divide (NDSU).

**Pathogen Material.** Cereal aphid species used were *R. padi* and *S. avenae* populations collected from a wheat production field at Thompson and Fargo, in 2015. Cereal aphids were placed in isolation cages in the greenhouse with non-infected wheat (WB-Mayville) plants. Oat plants infected with BYDV-PAV and CYDV-RPV (tested by ELISA test) were collected from wheat fields on the NDSU main-campus research plots, in Fargo, ND. *Rhopalosiphum padi* and *S. avenae* were placed in isolation cages with infected oat plants and allowed to feed for 12 to 14 days to acquire virus. Detached leaves harboring 8 to10 aphids were placed in isolation cages with healthy wheat (WB-Mayville) to rear aphid colonies carrying BYDV-PAV and CYDV-RPV. Periodic testing of the virus strains took place to ensure that the aphid colony was viruliferous.

**Field Experiment.** Research sites were established at Fertile, MN and Fargo, ND in 2016; and one site was established at Fargo in 2017. Trials were designed in a randomized complete block with a split-plot arrangement and replicated four times. Cultivar served as a main plot and infestation (presence and absence of cereal aphids) served as sub-plot. Plots were sown with an Almaco research planter (Nevada, IA) at a planting rate of 1.75 million seeds per hectare onto dryland. Plots were 1.68 m in length and 1.3 m wide (seven rows wide) with a row spacing of 19.05 cm. When plots were at tiller to stem elongation growth stages, 100-125 viruliferous cereal aphids (80% *R. padi* and 20% *S. avenae*) were randomly dispersed by placing plant tissue with feeding aphids at nine locations within the infested treatment plots. After 10 days, plots were sprayed with lambda-cyhalothrin to remove cereal aphids. Plots were rated for disease

incidence and severity at various points throughout the season. Plots were maintained with normal crop management practices including weed control, disease control and fertility requirements. Plots were harvested with Kincaid plot combine (Haven, KS). Plot weight and test weight were recorded.

**Greenhouse Experiment.** Evaluation of cultivars in the greenhouse was conducted in a complete randomized design with a factorial arrangement, six replicates, and repeated twice. Nine seeds of each cultivar were planted in 10.16 cm by 10.16 cm plastic greenhouse pots using Sunshine Mix potting soil. All pots were placed into individual insect containment cages measuring 15.24 cm by 91.44 cm and plots were thinned to five plants per pot.

For infested treatments, 10 adult cereal aphids (8 *R. padi* and 2 *S. avenae*) were placed onto the treatment plants at the tillering growth stage. Cereal aphids fed on plants for 10 days and then counted to determine the difference in aphid population numbers. Malathion was then used to terminate aphids. Once cereal aphids were terminated, insect cages were removed and plants were grown at 24 degrees °C under 12 light regime. All infested plants developed classic yellowing symptoms. At crop maturity, the number of spikes in each pot were counted and clipped at peduncle to spike attachment. Remaining shoot tissue (all plant material above soil line) was placed in a dryer at 60 degrees °C for 68 to 72 h. Plant material was then weighed and represented dry shoot mass. Flag leaf samples from each pot were collected and subjected to ELISA to detect the presence of BYDV-PAV and/or CYDV-RPV.

**Statistical Analysis.** For both the greenhouse and field studies, data was sorted according to market class (hard red spring wheat or durum) and analyzed using PROC GLIMMIX (SAS Institute, 2013). Homogeneity of variance was tested in greenhouse experiments using Levene's test (Levene, 1960). Least squared means were used to separate differences (alpha=0.95) for

aphid infestation. Fixed effects included infestation, cultivar and the interaction of cultivar by infestation. Replicate were included as a random effect.

Due to differences in yield potential and plant architecture of the cultivars, the variable effect sizes (relative differences from non-infested treatment) were created for number of spikes, dry shoot mass, and yield. Effect sizes were calculated using (1 - x/y)\*100 where *x* represents the infested value and *y* represented the non-infested value. Aphid number effect sizes were determined using (x-y) / y where *x* represented the final aphid count in a pot and *y* representing the initial amount of cereal aphids released into each infested pot. Data was analyzed using PROC GLIMMIX (SAS Institute, 2013) with replicate serving as a random effect.

# Results

**Field Results.** Disease incidence and severity was evaluated four to six times in each field trial. However, disease did not develop in any of the field sites during 2016 or 2017. Weather conditions at time of aphid release were not conducive for aphid reproduction and spread (high winds, large fluctuations in nighttime, and daytime temperatures). The seven-day average wind speeds after aphid release for 2016-Fargo, 2016-Fertile and 2017-Fargo were 11 Km/h, 18 Km/h and 10 Km/h, respectively. Also, wind gusts between 40 to 56 Km/h were recorded at each field location, and 2.8 to 4.3 centimeters of rain occurred within 7 d of aphid release in 2016. The combination of these environmental conditions likely contributed to high aphid mortality and prevented aphid dispersal in the plot. Therefore, agronomic data differences were likely attributed to other limiting factors (diseases, soil compaction, etc.) rather than BYD and these data will not be presented as it did not answer our research question - the impact of BYD on hard red spring wheat and durum grown in North Dakota.

Greenhouse Results. Data for all dependent variables were combined for both market classes with the exception of the number of spikes for hard red spring wheat (trials analyzed separately). For hard red spring wheat, aphid-infested pots had an average of four less spikes produced compared to the non-infested plots (Table 2.1). Similar reductions in the number of spikes were observed for durum (Table 2.2). Shoot mass for aphid-infested hard red spring wheat and durum was significantly reduced with average reductions around 15% (Tables 2.1 and 2.2). In addition, significant yield reductions were observed on plants that were infested with viruliferous cereal aphids, with average percent reductions of 23% and 24% for hard red spring wheat and durum, respectively (Tables 2.1 and 2.2). Varietal effect size differences were not observed for any dependent variables when comparing Barlow, Prosper and WB-Mayville (Table 2.3). Similar observations occurred in the durum varieties, with the exception of aphid populations differences (Table 2.4). Specifically, aphid populations were statistically higher on Carpio (274%) compared with Divide (158%).

Number of spikes				
Treatment	Trial 1	Trial 2	Dry shoot mass (g/pot)	Grain yield (g/pot)
Infested	13.50 b <sup>z</sup>	10.28 a	3.96 b	3.86 b
Non-infested	17.89 a	11.06 a	4.70 a	4.99 a
P > F	0.0004	0.02	0.0013	0.0001

**Table 2.1.** Least squares means comparing the effect of infestation on number of spikes, dry shoot mass, and yield for hard red spring wheat.

<sup>z</sup>Values accompanied by different letters within columns are statistically different from each other at the 95% confidence level.

**Table 2.2.** Least squares means comparing the effect of infestation on number of spikes, dry shoot mass, and yield for durum.

Treatment	Number of spikes	Dry shoot mass (g/pot)	Grain yield (g/pot)
Infested	7.7 b <sup>z</sup>	3.27 b	3.48 b
Non-infested	11.29 a	3.83 a	4.60 a
P > F	0.0004	0.02	0.008

<sup>z</sup>Values accompanied by different letters within columns are statistically different from each other at the 95% confidence level.

**Table 2.3.** Least squares means reflecting effect size differences among hard red spring wheat varieties for number of spikes, dry shoot mass, and yield for hard red spring wheat.

Market class	Aphids (%)	Spikes	Dry shoot mass (g/pot)	Grain yield (g/pot)
Barlow	3.58	0.15	0.21	0.16
Prosper	2.87	0.22	0.13	0.27
Mayville	2.50	0.07	0.08	0.24
P > F	0.1373	0.1813	0.3048	0.6545

**Table 2.4.** Least squares means reflecting effect size differences among hard red spring wheat varieties for number of spikes, dry shoot mass, and yield for durum.

Market class	Aphids (%)	Number of spikes	Dry shoot mass (g/pot)	Grain yield (g/pot)
Divide	1.58 b <sup>z</sup>	0.3784	0.1992	0.2842
Carpio	2.74 a	0.4142	0.1245	0.3067
P > F	0.0474	0.8496	0.4383	0.8208

<sup>z</sup>Values accompanied by different letters within columns are statistically different from each other at the 95% confidence level.

# Discussion

Several studies have evaluated the effect of BYDV and CYDV strains on yield and physiological components of small grains. However, most of the research has been conducted on soft red winter wheat and hard red winter wheat with limited studies performed on spring sown small grains (Hoffman and Kolb, 1997, Edwards et al., 2000). The majority of the spring sown small grains are grown in the northern Great Plains in areas where cereal aphids do not overwinter. As a result, the BYD risk may be lower than southern areas that predominately grow winter sown small grains. However, cereal aphid arrival into North Dakota has occurred earlier in the past five years elevating the risk for spring sown small grains. Our survey efforts over the past two years have indicated that higher incidence of cereal aphids have occurred in southeast and northwest North Dakota. Northwest North Dakota is leading producer of durum and economic loss assessments of diseases, such as BYD for this market class is needed. Greenhouse studies have shown that yield losses of 24% can occur in durum and a concerted effort in conducting additional field trials will help determine the BYD risk.

Greenhouse studies have shown that BYDV infection can disrupt normal plant growth limiting both root and shoot growth (Erion and Riedell, 2012; Hoffman and Kolb, 1997). Aeroponic studies conducted on winter wheat indicated severe reductions in root length in contrast shoot reductions were not as severe (Hoffman and Kolb, 1997). For one oat cultivar significant shoot reductions were observed where as one barley cultivar has no differences in shoot reductions due to BYDV infection in the greenhouse (Erion and Riedell, 2012). This study showed significant reductions in shoot mass of both hard red spring wheat and spring durum. These results from this study and previous research indicate that shoot response differences may

exist from BYDV infection among small grain market classes and future work could help elucidate differences.

Field studies on BYD have shown substantial reductions in yield and other physiological components of plant development. One study observed that the level of viruliferous aphid levels and early infestation negatively impacted yield (Perry et al., 2000). Same authors also suggested that environmental factors and strain selection of BYDV influence yield reductions of small grains. Another field study on spring wheat used insect cages to help improve cereal aphid survival (ie: protect aphids from predators) and recorded yield reductions as high as 62% (Riedell et al., 2003). In the field experiments evaluating three spring wheat varieties and two durum varieties, a high level of cereal aphids and BYD was not detected in the plots due to unfavorable weather conditions (high winds and rain) hindering aphid reproduction and dispersal.

When three spring wheat and two durum cultivars were evaluated in the greenhouse, significant reductions occurred in number of spikes, dry shoot mass and yield. However, statistical differences among hard red spring wheat varieties was not found. This could be due to the inherent susceptibility of hard red spring wheat since breeding efforts have focused on other fungal diseases, such as Fusarium head blight (*Fusarium graminearum*), leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis*), stripe rust (*Puccinia striiformis*), and fungal leaf spots (Andrew Friskop, *personal communication*). Similarly, statistical differences in agronomic variables among durum cultivars were not observed likely suggesting that breeding efforts have not focused on BYDV. Statistical differences in the amount of aphid increase was found between 'Carpio' and 'Divide'. A more thorough investigation may be warranted to determine if

antixenosis and antibiosis exist in these durum cultivars; similar to what has been done in triticale (*Triticosecale* x Whitt.) and wheat (Razmjou et al., 2012).

This research has established a baseline for assessing yield loss and plant loss risk when hard red spring wheat and durum grown in North Dakota are exposed to aphid vectoring BYDV. Evaluating more hard red spring wheat and durum varieties could help detect any varieties that are resistance or tolerant to cereal aphids and BYDV. Quantitative information about cultivars is critical for Extension publications and provide growers with updated cultivars facts on host plant resistance. Awareness of cultivar susceptibility to cereal aphids and BYDV will increase incentive to private breeders to eliminate extremely susceptible materials from their breeding programs. Finally, understanding the relationship between yield losses and BYD will help strengthen applied research and surveying efforts to detect the presence of cereal aphids vectoring BYD during the growing season.

### **Literature Cited**

- Bockus, W., De Wolf, E. D., and Todd, T. 2015. Effects of planting date, cultivar, and imidaclorprid Seed Treatment on Barley Yellow Dwarf Virus of Winter Wheat, 2012-2014. Plant Dis. Mgmt. 17, 122-127.
- D'Arcy, C. J., & Burnett, P. A. 1995. Barley Yellow Dwarf: 40 Years of Progress. St. Paul: APS Press. 9-29, 75-76, 203
- Edwards, M. C., Fetch, T. G., Schwarz, P. B., & Steffenson, B. J. 2000. Effect of barley yellow Dwarf Virus Infection on Yield. Plant Disease, 85, 202-207.
- Erion, G., & Riedell, W. (2012). Barley yellow dwarf virus effects on cereal plant growth and transpiration. Crop Sci., 52, 2794-2799.
- Levene, H. 1960. Robust tests for equality of variances pp. 278–292. In: I. Olkin, S. G. Ghurye, W. Hoeffding, W. G. Madow, and H. B. Mann (eds.), Contributions to probability and statistics: essays in honor of Harold Hotelling. Stanford Univ. Press, Stanford, CA.

NASS. 2016. 2016 state agriculture overview-North Dakota. USDA.

Painter, R.H. 1951. Insect resistance in crop plants. MacMillan, New York, 520 pp.

- Perry, K. L., Kolb, F. L., Sammons, B., Lawson, C., Cisar, G., & Ohm, H. 2000. Yield effects of barley yellow dwarf virus in soft red winter wheat. Am. Phytopath., 9, 1043-1048.
- Radcliffe, E., Hutchison, W., & Cancelado, R. (2017). Radcliffe's IPM world textbook. St. Paul: University of Minnesota.
- Ransom, J., et al. 2016. North Dakota hard red spring wheat variety trial results for 2016 and selection guide. Fargo: NDSU Extension Service.
- Razmjou, J., Mohamdi, P., Golizadeh, M., Hasanpour, M., & Naseri, B. (2012). Resistance of wheat lines to R. Padi under laboratory conditions. J. Econ. Entomol., 592-597.

SAS Institute. 2013. SAS Version 9.4. SAS Institute, Cary, NC.

Smith, C.M. 1989. Plant Resistance to Insects. Wiley, New York, 286 pp.