Coordination of Diagnostic Efforts in the Great Plains: Wheat Virus Survey and Modeling of Disease Onset

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Wheat is a staple grain crop for the majority of the world's human population. The United States accounted for 9% of global production in 2012, harvesting 61.8 million metric tons (t) (FAOSTAT 2014), and is often the world's largest wheat exporter, exporting half of its annual yield (Leifert 2013). Viruses are major perennial threats to wheat production in the Great Plains region of the United States. Accurate evaluations of crop loss are difficult to make, but researchers in Kansas estimated losses from wheat viral diseases to be ~1% annually (Appel et al. 2007). In 2011 and 2012, losses were estimated at 4.4 and 3.5%, respectively (Appel et al. 2015). Using a conservative estimate of a 1% yield loss on 20% of the current U.S. production of 60 million t (USDA-NASS 2014) for wheat valued at \$0.216/t (\$8/bu), U.S. farmers lose \$35 million per year due to cereal viruses.

Wheat Viruses, Vectors, and Management

Increasing global demand for wheat has encouraged growers to employ continuous cropping systems in response to the economic pressures related to the demand for cereals for the use of hay and grain. Continuous cropping has extended the "green bridge," the presence of green plants, including crops, volunteer crops, and grassy weeds between the harvesting of one crop and the planting of the next. Green bridge plants are important reservoirs of cereal viruses (Derksen et al. 2002; Edwards et al. 2011; Hansen et al. 2012). Therefore, cereal viruses are expected to increase in prevalence and remain an economic concern for growers. In addition, the discovery of two new viruses, *Triticum mosaic virus* (TriMV) (Seifers et al. 2008) and *Wheat mosaic virus* (WMoV; formerly High Plains virus [Seifers et al.

1997]), compound the threat of virus diseases in cereals. In 2006, TriMV was identified in varieties of wheat in Kansas resistant to *Wheat streak mosaic virus* (WSMV).

Both WMoV and TriMV cause patterns of chlorosis similar to WSMV. All three diseases are transmitted by the wheat curl mite (WCM; *Aceria tosichella* Keifer [Seifers et al. 2008]). These viruses represent a diverse phylogeny. WSMV is the type member of the genus *Tritimovirus* in the family *Potyviridae* (Stenger et al. 1998). TriMV is in the proposed genus *Susmovirus*, family *Potyviridae* (Fellers et al. 2009). TriMV has 23.2% identity to WSMV, and is most closely related (45.9%) to *Sugarcane streak mosaic virus* – strain AP (Fellers et al. 2009). The taxonomy of WMoV is complex, and it has recently been assigned to genus *Emaravirus* (Skare et al. 2006; Tatineni et al. 2014). WMoV is a multipartite, negative sense, single-stranded RNA virus with eight genomic segments (Tatineni et al. 2014). It has membrane-bound, spherical virions making it more similar to *Bunyaviridae* than *Potyviridae*.

Aphid-transmitted viruses, particularly the barley yellow dwarf (BYD) virus complex are common, cause additional disease losses, and can be easily misdiagnosed based on symptoms (Fig. 1) (Lister and Ranieri 1995). Both BYDV-PAV and CYDV-RPV belong to the family *Luteoviridae*, genus *Luteovirus* (Ali et al. 2014) and are transmitted in a circulative, nonpropagative manner (Gray and Gildow 2003).

Management of mite- and aphid-transmitted viruses is achieved primarily by managing the green bridge and through host plant resistance. In the southern Great Plains, however, reliance on grazed winter wheat makes eliminating the green bridge difficult (Tanaka et al. 2005). Although insecticides exist for aphid management, they are not always economical for use in cereals (Kennedy and Connery 2012). No effective pesticides (acaricides) are labeled for mites in wheat. Cereal crop breeders have worked for years to develop virus-, mite-, and aphid-resistant varieties, but have encountered many challenges. Varietal resistance provides partial protection from WSMV (Graybosch et al. 2009; Lu et al. 2011) and its vector, the WCM (Harvey et al. 1999), but this protection is limited by several factors. Resistant varieties tend to produce lower yields and the WSMV resistance gene currently deployed

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is not effective above 21°C (Bockus et al. 2001; Lu et al. 2011; Seifers et al. 2007). Additionally, WSMV-resistant varieties are not adapted to northern state climates, so are only grown in Texas, Oklahoma, Kansas, and Nebraska (Byamukama et al. 2011, 2013). Furthermore, virus and mite genomes can change rapidly and overcome host resistance (Harvey et al. 2003; Seifers et al. 2007). Despite these challenges, researchers continue to pursue the development of new, resistant varieties (Rupp et al. 2014).

National Plant Diagnostic Network

Accurate identification of wheat viruses requires diagnostic testing in a laboratory. The National Plant Diagnostic Network (NPDN) was created in 2002 with support from the United States Department of Agriculture National Institute of Food and Agriculture (USDA-NIFA). It is a consortium of diagnostic laboratories at land grant universities and state departments of agriculture that has strengthened partnerships among diagnosticians, university cooperative extension personnel, and regulatory officials (Stack et al. 2006, 2014). The NPDN is composed of five regions: the Northeastern Plant Diagnostic Network (NEPDN), the North Central Plant Diagnostic Network (NCPDN), the Southern Plant Diagnostic Network (SPDN), the Great Plains Plant Diagnostic Network (GPDN), and the Western Plant Diagnostic Network (WPDN).

NPDN is an important part of the U.S. effort to ensure the security of the nation's plant resources (Stack et al. 2006, 2014). Importantly, the NPDN helps inform cooperative extension personnel, researchers, and regulatory officials of emerging threats posed by plant pests and pathogens. The Center for Environmental and Regulatory Information Systems (CERIS) at Purdue University houses and supports the NPDN National Data Repository, which contains the diagnostic results of NPDN laboratories.

The NPDN has created a secure, up-to-date national archive for diagnostic records from participating laboratories throughout the United States and U.S. territories. Authorized specialists can consult the repository to monitor diagnostic trends for common pests and outbreaks of new or unusual pests and diseases in real time.

Diagnosticians across the Great Plains have expressed concern about the accuracy of identifying virus species since the detection of two new mite-transmitted wheat viruses. Symptoms for WMoV and TriMV are virtually indistinguishable from WSMV symptoms. Additionally, BYD symptoms are nearly indistinguishable from abiotic stress or nutrient deficiencies (Bockus et al. 2010). During a meeting in 2007 of the GPDN, diagnosticians discussed the

increasing incidence of virus infections in wheat. In response to concerns about accurate diagnosis of virus diseases of cereals and the impact of multiple viral infections on the crops, diagnostic labs of the GPDN began a region-wide collaboration to address the problem (Burrows et al. 2009). The approach was four-pronged: (i) conduct a survey in the Great Plains for the five wheat viruses from 2008 to 2010; (ii) develop and implement a multistate, formal agreement among regulatory agencies to allow diagnosticians to share their diagnostic results with other diagnosticians and regulatory officials; (iii) use the information and analytical tools in the NPDN National Data Repository to understand the timing of detectable wheat virus onset; and iv) use a model based on degree-days as an indicator of optimal timing to conduct sampling and processing during the survey. In this paper, we describe results of this survey and the multistate collaborations enabling new insights into the dynamics of wheat virus pathosystems in the Great Plains.

The Great Plains Wheat Virus Survey and Sample Collection

This project was a three year collaboration (2008 through 2010) among agricultural extension agents, extension specialists, state departments of agriculture, federal regulators, crop advisors, and NPDN laboratories in nine states: Colorado, Kansas, Montana, Nebraska, North Dakota, Oklahoma, South Dakota, Texas, and Wyoming (Burrows et al. 2009). Samples of winter and spring wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) were collected and submitted to the NPDN laboratories. All samples had virus-like symptoms, were part of routine survey activities, and/or were solicited from county extension agents and crop advisors. The samples were not collected according to standardized or random sampling plans, but usually from symptomatic plants. More than 95% of the samples were from winter wheat. Less than 5% of samples were from barley and spring wheat submitted from Montana, North Dakota, and South Dakota. Sample collection was not randomized and collection criteria were not consistent from state to state. Samples were collected and submitted to NPDN diagnostic laboratories based on perceived grower need and/or surveys conducted by individual cooperating states.

Virus Detection

All wheat samples received in GPDN diagnostic laboratories were tested for five viruses: WSMV, TRIMV, WMoV, *Barley yellow dwarf*

Fig. 1. Symptoms of *Wheat streak mosaic virus* (A), *Triticum mosaic virus* (B), and *Wheat mosaic virus* (C) are very similar. *Barley yellow dwarf virus* symptoms are different (D). Virus infections can be confused with nutrient deficiency and other environmental stresses (E).

virus-PAV (BYDV-PAV), and *Cereal yellow dwarf virus-RPV* (CYDV-RPV) using indirect ELISA (Agdia Diagnostics, Elkhart, IN). An agreement among diagnostic laboratories was created to standardize wheat virus testing throughout the GPDN laboratories. Samples were individually processed according to the instructions of the manufacturer of the ELISA kits. Each kit consisted of a single non-coated ELISA plate (96-well formatted in 8-well break-apart 'strips') for each of the five viruses, appropriate positive and negative controls, and reagents required for the assays including antibodies and buffers. Absorbance at 405 nm was measured using an ELISA plate reader, which varied by manufacturer and model among diagnostic laboratories. Samples were considered positive if the absorbance value of the sample exceeded twice the absorbance value of the negative control (i.e., healthy wheat). If the ELISA was inconclusive the sample was considered negative. TriMV test results from North Dakota and South Dakota in 2009 were excluded from the data set due to difficulties with the TriMV ELISA. However, end-point PCR and BLAST analysis confirmed the presence of TriMV in both states. At the time, PCR tests

were nonstandardized, using a variety of primers derived from the currently known sequences from GenBank. Infected plant tissues were preserved by lyophilization and storage at -80°C for use in future research projects (Rogers 2011).

Survey Data Reporting

A total of 2,456 samples were collected and processed during the course of the survey (Table 1). Data were expressed as the percentage of samples infected compared with the number of samples processed for each county for each year. The survey sample set reported in this paper is biased due to the nature of sample collection. Therefore, it is difficult to draw conclusions regarding year-to-year variation in virus frequency since sample numbers were largely tied to survey efforts in individual states. For example, in 2009 in Montana, a cooperative agricultural pest survey of small grains was performed with the Montana Department of Agriculture and APHIS-PPQ. This resulted in more small grains samples submitted for virus diagnosis than would normally come into the diagnostic laboratory.

Table 1. Incidence (%) of wheat samples testing positive for *Wheat streak mosaic virus*, (WSMV), *Wheat mosaic virus* (WMoV), *Triticum mosaic virus* (TriMV), *Barley yellow dwarf virus* (BYDV)-PAV, and *Cereal yellow dwarf virus* (CYDV)-RPV, and coinfections during a survey of Great Plains states, 2008 to 2010

Percent of samples testing positive using ELISA ^{a,b}											
State	n	Mite transmitted			Aphid transmitted		Coinfections				
		WSMV	WMoV	TriMV	BYDV-PAV	CYDV-RPV	WSMV + WMoV	WSMV + TriMV	WMoV + TriMV	All mites ^c	All aphids ^d
2008											
Colorado	51	61	10	10	10	4	8	8	0	0	0
Kansas	53	62	38	30	6	2	15	21	13	8	0
Montana	23	43	9	0	9	0	9	0	0	0	0
Nebraska	66	39	8	27	5	3	8	18	5	5	2
North Dakota	44	40	12	0 ^b	2	0	9	0	0	0	0
Oklahoma	93	27	30	6 ^b	16 ^b	3 ^b	16	4	3	3	2
South Dakota	96	28	7	2	3	0	7	2	1	1	0
Texas	307	83	41	57	14	2 ^b	37	53	28	26	0
Wyoming	21	38	19	24	0	0	5	10	10	0	0
Average		47	19	17	7	2	13	13	13	5	0
Total	754										
2009											
Colorado	145	50 ^b	24 ^b	21 ^b	32 ^b	8 ^b	70	67	46	6	5
Kansas	50	14	0	2	10	0	14	16	2	0	0
Montana	149	29	4 ^b	7	1	2	3	5	1	1	1
Nebraska	97	14	16	44	8	14	31	57	60	3	1
North Dakota	95	52	12	.	6	9 ^b	63	.	.	.	0
Oklahoma	77	49	17	18 ^b	65	4	66	68	36	0	4
South Dakota	44	27	14	.	5	.	41	.	.	.	.
Texas	320	44	4	14	20	0	48	58	18	2	0
Wyoming	21	10	19	0	5	0	76	14	10	0	0
Average		32	12	20	17	4	46	46	29	2	1
Total	998										
2010											
Colorado	118	44	14	25	23	11	5	20	2	2	3
Kansas	62	13	0	8	18	0	0	5	0	0	0
Montana	61	48	4 ^b	0 ^b	0 ^b	0 ^b	2	0	0	0	0
Nebraska	122	11	6	47 ^b	9	0	0	0	0	0	0
North Dakota	35	60	15	0	8	0	15	0	0	0	0
Oklahoma	36	33	22	11	50	8	17	11	11	11	8
South Dakota	67	13	4	4	9	1	1	0	0	0	1
Texas	170	26	1	8	13	6	1	8	1	1	3
Wyoming	33	6	0	0	0	0	0	0	0	0	0
Average		28	7	11	14	3	4	5	1	1	2
Total	704										

^a A period (.) indicates difficulties with the ELISA for detecting virus; data not shown.

^b Percentage based on total number of samples per state (n) unless noted here. In 2008, North Dakota (n = 44) processed 41 TriMV samples; Oklahoma (n = 93) processed 89 TriMV, 90 BYDV-PAV and 90 CYDV-RPV samples; and Texas (n = 307) processed 257 BYDV-RPV samples. In 2009, Colorado (n = 145) processed 144 WSMV, 100 WMoV, 98 TriMV, 92 BYDV-PAV, and 91 CYDV-RPV samples; Montana (n = 149) processed 148 WMoV samples; North Dakota (n = 95) processed 93 CYDV-RPV samples; Oklahoma (n = 77) processed 76 TriMV samples. In 2010, Montana (n = 61) processed 46 samples for WMoV and 47 samples for TriMV, BYDV-PAV, and CYDV-RPV; Nebraska (n = 35) processed 15 samples for TriMV.

^c Percentage of plants tested positive with ELISA for mite-transmitted viruses (WSMV, WMoV, and TriMV).

^d Percentage of plants tested with ELISA that were positive for aphid-transmitted viruses (BYDV-PAV and CYDV-RPV).

Despite these limitations, this dataset provided confirmation that WSMV was the most prevalent, meaning the most commonly occurring virus as quantified by incidence, detected in every state. Overall, 36% of sample date and location combinations tested positive for WSMV with an incidence ranging from 6 to 83% among states (Table 1). TriMV was the next most prevalent virus detected with incidence averaging 16% among states and ranging from 0 to 57%. Thirteen percent of samples tested positive for WMoV (range 0 to 41%) and 13% tested positive for BYDV-PAV (range 0 to 65%). CYDV-RPV was the least prevalent virus detected (3% overall average, range 0 to 14%). Forty-six percent of samples had no detectable viruses and 30% of samples tested positive for only one virus. Twenty-one percent of samples were coinfecting, meaning they tested positive for two or more viruses (Tables 1 and 2).

Both WMoV and TriMV were found in all nine states during the course of the survey. TriMV had not been detected outside of Kansas prior to this survey. As specified by the NPDN Chain of Communication-Chain of Custody Standard Operating Procedure, State Plant Regulatory Officials (SPROs) and State Plant Health Directors

(SPHDs) were notified in each state by the NPDN diagnostician, upon the first positive results of these new viruses. From the widespread nature of this newly identified virus, it appears to have been present but unrecognized in U.S. cropping systems for an extended period of time. TriMV was therefore not designated as a “regulated” pest by APHIS-PPQ. If a pest is designated as “regulated,” it is not generally established in the United States, is frequently intercepted from commodities at U.S. ports of entry, and has the potential to cause serious economic or environmental damage in the United States. (USDA-APHIS 2012). Lack of recognition of TriMV as a distinct virus may have been due to the similarity of TriMV symptoms to WSMV. The current survey results also confirmed that WSMV was more common than TriMV and that TriMV was found primarily in coinfections with WSMV, rather than as a single infection (Byamukama et al. 2013) (Table 1). Since the inception of this survey, others have also identified TriMV in samples outside of Kansas and its occurrence appears to be widespread (Byamukama et al. 2013).

We also observed that TriMV and WMoV were highly prevalent (7 to 20% of samples on average per year), and often present in coinfections with other WCM-transmitted viruses (Table 1). All three WCM-transmitted viruses were rarely found in a single plant sample (an average of 1 to 5% of samples per year; Table 2).

Aphid-transmitted viruses in the BYD complex (BYDV-PAV and CYDV-RPV) were infrequent. Possible reasons for this include: (i) a less frequent occurrence in the Great Plains than mite-transmitted viruses, (ii) growers and agricultural professionals were more confident in their ability to recognize this virus disease and therefore did not submit samples for diagnosis, or (iii) symptoms of BYD complex diseases are easily mistaken for nutrient deficiencies. During the years of the study BYD was a minor disease of wheat. In 2014 there were outbreaks of BYD in Idaho, North Dakota, and Minnesota (Friskop 2014; Marshall and Rashed 2014).

Development of a Degree-Day Model for Predicting Onset of Detectable WSMV

WSMV records and data analysis tools from the NPDN National Data Repository were used to develop a degree-day model for estimating the onset of detectable WSMV in field samples. Degree-day map layers were imported into the NPDN National Data Repository analysis system from the Oregon State University Integrated Plant Protection Center’s (OSU IPPC) weather-based online decision support platform via the website <http://uspest.org/wea> (Oregon State University 2012). For each sample, cumulative degree-days base 0°C (CDD0°C) were calculated from January 1 each year to the date of sample collection. CDD0°C was selected to be consistent with wheat phenology (growth stage) models postulating that phenology may be correlated with detectable WSMV. Wheat phenology models were selected that are well documented and currently in use (Hay and Kirby 1991; Karow et al. 1993; Keppeler et al. 1988; McMaster and Wilhelm 1997; McMaster and Smika 1988; Miller et al. 2001; Porter and Gawith 1999; Saiyed et al. 2009; Streck et al. 2003; Wang and Engel 1998). Degree-day values were calculated using the temperature-based single sine curve method with no upper threshold (Baskerville and Emin 1969). The start date of January 1 was selected because McMaster and Smirka determined that winter wheat phenology degree-day models that started at January 1 more accurately estimated the correct growth stage than models that started at sowing or emergence for winter wheat since vernalization and other synchronization mechanisms in winter wheat reduced the variability contributed by fall planting dates particularly in northern latitudes (McMaster and Smika 1988; Streck et al. 2003; Wang and Engel 1998). Since sample location was collected at the county resolution but not subcounty, the geographic center of the county (county centroid) was used as the sample location. The OSU IPPC’s weather-based decision support platform was used to generate a CDD0°C value for the nearest weather station to the county centroid on the date of collection for each sample. The system’s weather database of over 16,000 weather stations from numerous public networks was obtained from Mesowest at the University of Utah and from numerous grower-led weather networks (University of Utah 2012). After ingestion of the raw weather data, the decision support platform performed a rigorous

Table 2. Incidence (%) of single and coinfection of wheat plants during a survey of Great Plains states, 2008 to 2010

		Number of viruses coinfecting each sample					
		0	1	2	3	4	5
2008	<i>n</i> ^a	Percent of samples infected (%) ^b					
Colorado	51	22	63	16	0	0	0
Kansas	53	8	55	30	8	0	0
Montana	23	48	43	9	0	0	0
Nebraska	66	47	29	20	5	0	0
North Dakota	44	57	34	9	0	0	0
Oklahoma	93	49	28	17	3	2	0
South Dakota	96	68	23	7	1	0	0
Texas	307	8	23	37	28	4	0
Wyoming	21	43	33	24	0	0	0
Average		39	37	19	5	1	0
		Number of viruses coinfecting each sample					
		0	1	2	3	4	5
2009	<i>n</i>	Percent of samples infected (%) ^b					
Colorado	145	33	41	14	11	0	1
Kansas	50	82	15	3	0	0	0
Montana	149	67	24	7	1	0	0
Nebraska	97	36	41	17	6	0	0
North Dakota	95	39	48	8	4	0	0
Oklahoma	77	10	34	48	8	0	0
South Dakota	44	66	23	11	0	0	0
Texas	320	46	34	14	4	2	0
Wyoming	21	76	14	10	0	0	0
Average		49	29	18	4	0	0
		Number of viruses coinfecting each sample					
		0	1	2	3	4	5
2010	<i>n</i>	Percent of samples infected (%) ^b					
Colorado	118	26	38	29	6	1	0
Kansas	62	69	24	5	2	0	0
Montana	61	52	48	2	0	0	0
Nebraska	35	57	40	3	0	0	0
North Dakota	122	34	49	16	1	0	0
Oklahoma	36	17	53	19	11	0	0
South Dakota	67	72	25	1	1	0	0
Texas	170	61	26	10	2	1	0
Wyoming	33	94	6	0	0	0	0
Average		54	34	9	3	0	0

^a Number of cereal plant samples tested from each state for *Wheat streak mosaic virus*, *Wheat mosaic virus*, *Triticum mosaic virus*, *Barley yellow dwarf virus-PAV*, and *Cereal yellow dwarf virus-RPV*.

^b Data expressed as percent of samples from each state tested by ELISA that were infected with 0 to 5 viruses in a single sample.

automated quality control system that scans for and corrects errors and missing values using distance and elevation weighted regression estimation of values from nearby weather stations (unpublished data).

Degree-day values for the sample collection dates in 2008, 2009, and 2010 were plotted for each sample to estimate a threshold value for onset of detectable WSMV (Fig. 2). Only 30 of the 435 samples which tested positive for WSMV were collected below 700 CDD $^{\circ}$ C. It is possible that prior to reaching the 700 CDD $^{\circ}$ C threshold there is a phenological development in the host plant or vector that prevents virus titer from multiplying to detectable levels in the host. Assuming a range of 600 to 800 CDD $^{\circ}$ C between fall planting and January 1, the growth stage of winter wheat at 700 CDD $^{\circ}$ C after January 1 is between the “6-leaves-expanded-with-tillering-ongoing” growth stage (Feekes wheat model growth stage 6 or Zadocks wheat model growth stage 31) and “flag-leaf-sheath-extended” growth stage (Feekes wheat model growth stage 10 or Zadocks wheat model growth stage 45) (Karow et al. 1993; Keppler et al. 1988; Large, 1954; Miller et al. 2001; Zadocks et al. 1974). This is when the plant is in late vegetative stages and shifting its metabolic processes toward reproductive stages (heading). The development and growth rate of wheat organs (e.g., leaves, tillers, spikelets, and inflorescences) changes as do minimum, optimum, and maximum base temperatures when the wheat plant transitions from the vegetative to reproductive stages (Porter and Gawith 1999; Slafer and Rawson 1995; Streck et al. 2003). It is not known if the mechanism of symptom development or virus titer is affected by this shift.

NPDN as a Tool for Disseminating Information: 2010 Wheat Virus Alerts

A regional diagnostic data-sharing agreement was developed among GPDN diagnosticians, state regulatory officials, SPHDs, and SPROs that allowed GPDN diagnosticians to share diagnostic records. All SPROs from GPDN states signed this agreement allowing GPDN diagnosticians to share the diagnostic results with all GPDN SPROs, SPHDs, and diagnosticians. This is the first time a regional diagnostic data-sharing agreement was developed among NPDN diagnosticians and state and federal regulatory officials to allow this degree of sharing. Prior to this, diagnosticians were not allowed to share diagnostic results with each other between states. In 2008 and 2009, data were shared on weekly teleconference calls with diagnosticians.

During 2009, a degree-day threshold of 700 CDD $^{\circ}$ C was proposed to indicate the first detection of WSMV positive samples each year. The threshold was based on the results from the 2008 and 2009 diagnostic samples. In 2010, county resolution maps of observed

WSMV-positive and WSMV-not-detected counties and the continuous variable CDD $^{\circ}$ C values were generated using the NPDN analysis tools in the NPDN National Data Repository (Fig. 3). A county was considered WSMV positive if any sample results from the county were WSMV positive to date since January 1 of the same year. Similar county resolution maps were generated for the other four wheat virus diseases surveyed, but were not integrated into a degree-day map. These multistate maps were distributed by e-mail to the diagnosticians, SPROs, and SPHDs as semimonthly updates during April, May, June, and July 2010 along with a text summary of diagnostic results over the prior two weeks (Supplementary Fig. S1).

The 700 CDD $^{\circ}$ C model was able to give general guidance on when to expect the first positive WSMV samples to originate from a local area (Fig. 3). The first positive WSMV county detections (red outlined counties on May 28, 2010) were observed within 2 weeks after CDD $^{\circ}$ C values were 700 to 900 (the pale blue locations on the map indicated by red arrows and circles on May 14th insert). When CDD $^{\circ}$ C was 700 to 900 and onset of disease was not observed, it may have been due to an absence of the vector or the virus. Based on these observations, a more rigorous statistical analysis was performed and is presented in the case-control section below.

Sample collection patterns for WSMV in 2010 were different than in 2008 and 2009. Two discrete populations were observed in 2010 when CDD $^{\circ}$ C was plotted by date and diagnostic test result for samples collected prior to 2,200 CDD $^{\circ}$ C (Fig. 2). For any CDD $^{\circ}$ C value in 2010, positive samples were detected before not-detected samples for all CDD $^{\circ}$ C values with positive WSMV detections. In addition, 2010 data had a smaller range of sample dates than the previous years. It is possible that the wheat virus updates influenced the timing of sample collection and processing, shortening the sampling period to be closer to the time of the observed first occurrence of WSMV positive test results.

Case-Control Analysis

In 2012, a case-control analysis was conducted to test the relative value of using the 700 CDD $^{\circ}$ C threshold compared with other CDD $^{\circ}$ C thresholds. The NPDN National Data Repository held diagnostic results for samples collected from the GPDN from 2006 to 2011 at the time that the case-control analysis was conducted in 2012. In the case-control study, replicated samples were pooled as one sample for each location, date, and test result combination to reduce the bias when many samples were collected at one sample point, since only one sample was collected from many locations. In the case-control

Fig. 2. Plot of cumulative degree-days base 0° C (CDD $^{\circ}$ C) calculated from January 1 until the wheat sample date compared with *Wheat streak mosaic virus* (WSMV) diagnostic test result (confirmed positive or not-detected) for sample collection dates in 2008, 2009, and 2010 for winter wheat in the Great Plains states, United States, in from 2008 to 2010. Only 30 of the 435 samples which tested positive for WSMV were collected above 700 CDD $^{\circ}$ C.

analysis, there were 849 unique degree-day-location-sample-date combinations for WSMV, 414 (49%) samples tested as WSMV not-detected and 435 (51%) samples tested as WSMV positive.

The association between degree-day accumulation and virus incidence was examined with the case-control design method (Hughes et al. 1999) and discussed at length by others (Hughes and McRoberts 2014; Madden et al. 2007; Yuen and Hughes 2002). In this study, our objective was to test the potential for using CDD0°C as an indicator of final ELISA outcome for WSMV for an individual plant sample. In other words, could degree-day accumulation provide a simple indicator of ELISA outcome that can be used as a prior proxy for, or in addition to, diagnostic laboratory tests? If so, then the degree-day value measured by CDD0°C could inform sample collectors as to the most likely first date to detect virus-positive samples, improving survey efficiency.

To examine the accuracy of degree-day based predictions, it is necessary to have an independent, “gold standard” classification of

samples to compare with predicted virus status. The WSMV ELISA test result was used as the “gold standard” for the presence of WSMV in the plant sample.

To determine the CDD0°C threshold with the highest probability of obtaining a virus-positive ELISA test from a field sample, the CDD0°C for each sample was partitioned at different threshold values in 100 CDD0°C intervals starting at 500 CDD0°C (Hughes et al. 1999). Each sample was classified as either having accumulated fewer than (<) or greater than or equal to ($\geq$) the threshold CDD0°C at values of 500, 600, 700, 800, 900, and 1,000 CDD0°C. For each cumulative degree-day threshold, a 2 × 2 outcome table was constructed in which every sample was cross-classified by whether it was a “case” (V+) or “control” (V-).

A threshold of 700 CDD0°C provided the largest amount of formal information concerning the virus status of test samples added by a sample’s CDD0°C value (Table 3). This result retrospectively concurs the use of the 700 CDD0°C threshold selected from the

Fig. 3. Example of the relationship over time between cumulative degree-days base 0°C (CDD0°C) since January 1 and the first county positive *Wheat streak mosaic virus* (WSMV) test result of the year. Arrows indicate areas between 700 and 900 CDD0°C since January 1, on May 14, 2010 predicting onset of WSMV symptoms by May 28, 2010. Red circles indicate first observed WSMV positive test results on May 28, 2010 by county in predicted areas.

Table 3. Contingency table (number of samples) and resulting likelihood ratios in a case-control analysis for a threshold of 700 cumulative growing degree-days base 0°C (CDD0°C) starting January 1 for *Wheat streak mosaic virus* (WSMV) ELISA test results during 2008 to 2010 in the Great Plains, United States. Each sample represents a unique county-date-year combination

ELISA results	Observed no. samples		Total no. samples	Likelihood ratio
	CDD0°C <700	CDD0°C >700		
WSMV not detected	56	357	413	Case LR+ 1.077 Case LR- 0.509
WSMV positive	30	405	405	Control LR- 0.928 Control LR+ 1.966
Totals	85	762	848	

2008–2009 diagnostic results and distributed during the 2010 wheat virus alerts.

Samples collected when CDD0°C were less than 700 were twice as likely to be virus negative and half as likely to be virus positive. This result supports the hypothesis that it is most productive to sample when CDD0°C are at or above 700 if the goal is to detect positive results. While these may appear to be relatively modest gains in accuracy at the level of an individual sample, they may represent significant gains in efficiency obtained by better timing of sample collection when considered at the scale of all samples collected over the entire region over multiple years.

Case-study methodology was originally applied to clinical diagnostic testing of individual patients in medical clinics. Its use in plant health was adapted to plant populations in the field, not to individual plants. This is the first application of a case-control design to plant diagnostic clinic results for individual plants and uses a multistate plant-diagnostic data set.

Even after taking into account degree-day accumulation, the results indicate the possible effect of other factors such as plant variety, planting date, and prior field history. These factors were not analyzed and could also influence the odds that the sample test result will be WSMV positive or WSMV not-detected. There was no standardization in sampling strategy with regards to host variety, host susceptibility, prior WSMV or WCM presence for the collection site or neighboring collection sites, water availability, plant fertility, weed control, or presence of a green bridge. These factors are known to impact phenology stage and also may be important in determining ELISA results at or above 700 CDD0°C (McMaster and Smika 1988; Slafer and Rawson 1995). Degree-day calculations started on January 1 to minimize the additional impacts of planting date, winter minimum temperatures, winter snow cover due to vernalization, and other synchronization factors. The degree-day model is likely an estimate of the stage of wheat phenology when symptoms are first expressed (Karow et al. 1993; Keppler et al. 1988; Miller et al. 2001; Porter and Gawith 1999; Streck et al. 2003; Wang and Engel 1998). Future work should explore likelihood ratios after dividing the data set into geographic subregions, with similar planting dates, cultivars, and other regional factors. These may have a practical effect on the likelihood ratios for WSMV in individual plants, particularly for virus-positive outcomes.

Perspectives Since the Survey

This project was a prime example of taking a common concern, engaging a network of collaborators, and tapping diagnostic resources to rapidly learn important information about an emerging pathosystem. Results were used to leverage over \$5 million in funding for additional research, extension, and educational efforts that will directly impact the profitability of farms in the Great Plains. Despite the known inconsistent methods of data collection, the initial survey provided a useful starting point for a larger effort. Since this survey, research scientists and extension personnel have cooperated on a number of wheat virus projects to understand the pathosystem and mitigate disease risk for growers. This cooperation has culminated in a six-state project supported by USDA-NIFA to develop a risk model for disease throughout the Great Plains, from Texas to Montana. Core experiments being conducted in Montana, Nebraska, and Texas include a variety trial, planting date study, and monitoring of important green bridge hosts for the presence of virus and potential mite vectors. These data along with weather data and the further ongoing work on the contribution of hail to subsequent disease, will feed into a risk model that growers can use (Byamukama et al. 2011, 2013).

The wheat virus system also is being used in educational modules for K-12 education and outreach. We developed a series of interactive approaches that have been used at outreach presentations including the use of “gigaballs” as virus models for young children. We have also conducted grower surveys to identify barriers to the adoption of disease management recommendations, such as avoiding green bridges, and to evaluate changing perceptions and grower practices. Some of the most important barriers to eliminating the green bridge are social and marketing pressures, not an understanding of

the science. The results of these ongoing projects underscore the importance of science-based extension education of growers and the need for continuing education of new growers, especially as farming operations become larger and more complex. The diagnostic network results were a key component of that educational system.

Conclusion

This paper summarizes an initial effort to learn more about an emerging complex of wheat viruses in the Great Plains using the resources of the NPDN. Our work constitutes the first signed formal diagnostic data-sharing agreement between NPDN and its regulatory stakeholders. This is also the first paper to apply case-control methodology to clinical plant pathology using plant-diagnostic laboratory data. This proof-of-concept effort highlights the potential of the NPDN and the NPDN National Data Repository to develop useful information about an emerging disease. We learned that WSMV, WMoV, and TriMV were found in all nine Great Plains states and WSMV was the most prevalent. Additionally, TriMV and WMoV were usually found in coinfections with WSMV, but seldom was a plant infected with all three. Aphid-transmitted viruses in the BYD complex (BYDV-PAV and CYDV-RPV) were relatively infrequent. In 2010, semimonthly maps and tables indicating the presence of these wheat virus diseases demonstrated a potential for influencing efficiency by timing sample collection.

Samples collected when CDD0°C were less than 700 were twice as likely to be virus negative and half as likely to be virus positive. Sampling and diagnostics are resource-intensive processes requiring significant investments of human capital, supplies, and reagents. The gain in likelihood of a positive WSMV test result based on time of sampling has potential to improve sampling and testing efficiency and reduce these costs. The information in the present study would have been very difficult to obtain without the resources of the NPDN. The potential for the resources of the NPDN and its National Data Repository to advance scientific discovery in the context of new disease and pest outbreaks is promising.

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