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Agroecological and environmental factors influence Barley yellow dwarf viruses in grasslands in  
the US Pacific Northwest

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## Highlights

- 1. BYDV was detected at all CRP and prairie remnants with an overall infection of 46%.
- 2. BYDV-SGV and BYDV-PAV were the only two B/CYDV species encountered.
- 3. Sampling time and plant species were most important in predicting BYDV prevalence.
- 4. BYDV was more prevalent in perennial compared to annual grasses.
- 5. BYDV prevalence increased with grain crop cover within 1 km radius of habitat patch.

## Abstract

Plant pathogens can play a role in the competitive interactions between plant species and have been understudied in native prairies, which are declining globally, and in Conservation Reserve Program (CRP) lands in the United States. *Barley/Cereal yellow dwarf virus* (B/CYDV) are among the most economically important disease-causing agents of small grain cereal crops, such as wheat, and are known to infect over 150 *Poaceae* species, including many of the grass species present in prairies and CRP lands. Field surveys of *Poaceae* species were conducted in endangered Palouse Prairie and CRP habitats of southeastern Washington and adjacent northern Idaho, USA from 2010-2012 to examine for the presence of B/CYDV among plant hosts and aphid vectors. Viral species were identified via cloning and sequencing. Landscape, soil and climate data were retrieved from USDA-NASS and USDA-NRCS databases. Analyses were conducted to examine effects of diverse agroecological and environmental factors on virus prevalence. A total of 2,271 grass samples representing 30 species were collected; 28 of these were infected with BYDV in at least one location. BYDV infection was detected at every CRP and prairie remnant sampled, with an overall infection of 46%. BYDV-SGV and BYDV-PAV were the only two B/CYDV species encountered, with BYDV-SGV being more prevalent. Sampling time (season) and host plant identity were the main variables explaining variation in virus prevalence among sites. BYDV was more prevalent in perennial compared to annual grass species. Aphids were encountered only once suggesting non-colonizing aphids, potentially from

neighboring cereal fields, are responsible for disease spread in these habitats. BYDV prevalence increased in sampled habitats as cereal crop cover increased within a 1-km radius of a habitat patch. Results demonstrate moderate to high and persistent prevalence of BYDV in an endangered grassland habitat. Species composition and susceptibility to pathogens should be considered when creating seed mixes for CRP sites, especially in relation to agricultural crops and diseases in a region. Future work exploring host abundance, competence and habitat utilization by vectors is required to fully elucidate BYDV ecology and epidemiology in grassland habitats.

**KeyWords:** Conservation Reserve Program; ; ; ; , Palouse Prairie, Luteoviridae, virus ecology, virus grass hosts, virus epidemiology

## 1. Introduction

Much of the landscape that was once dominated by native grasslands throughout the world has been transformed into agricultural production or developed as urban spaces. Grasslands are globally declining, leading to many of them becoming threatened and/or endangered habitats. For example, southeastern Washington and adjacent northern Idaho, USA, are home to the critically endangered Palouse prairie ecosystem which has historically been dominated by bunchgrasses, such as Idaho fescue (*Festuca idahoensis* Elmer) and bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) Á. Löve] (Noss et al., 1995; Lichtardt and Moseley, 1997; Donovan et al., 2009; Scheinost et al., 2009). The prairie that once dominated the Palouse landscape is now reduced to small remnants (< 2 hectares) located in areas that could not be farmed and are mostly privately owned (Hanson et al., 2008; Donovan et al., 2009; Looney and Eigenbrode, 2012). Less than 0.1% of the historical prairie habitat remains (Noss et al., 1995; Black et al., 2000). Preservation of these natural habitats is important not only to retain the unique species that occur in them, but also to preserve the ecosystem services they provide, such as mitigation of greenhouse gases and habitat for pollinators and predatory insects. One important and understudied factor that influences the integrity of these habitats is plant pathogen prevalence.

Yellow dwarf disease is one of the most economically important diseases of cereal crops globally (Plumb, 1983), affecting wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) and many other *Poaceae* species worldwide (D'Arcy, 1995). It is caused by a complex of viruses belonging to the family *Luteoviridae* referred to as *Barley* or *Cereal yellow dwarf virus* (B/CYDV) (Fauquet et al., 2005). B/CYDV are transmitted exclusively by aphids in a persistent circulative manner (Gildow, 1993), they are not transmitted mechanically or through seed. At

least 25 species of aphids are reported as vectors of B/CYDV (Halbert and Voegtlin, 1995). This pathosystem has served as a model when examining the dynamics of insect-borne plant pathogens between agricultural and adjacent habitats (Jones, 2004; Vincent et al., 2014) and among species in pastures and nonmanaged grasslands (Coutts and Jones 2002; Borer et al., 2007; Power et al., 2011; Jones 2013).

In one well-studied nonmanaged system, BYDV infection was prevalent among many of the grass species in California grasslands (Grafton et al., 1982). A survey in 1990 revealed that 37 of 56 cool-season grass species were infected with BYDV in California (Griesbach et al., 1990). BYDV has also been shown to play an important role in the competitive dynamics of native and invasive grasses in these communities (Malmstrom et al., 2005a; Malmstrom et al., 2005b; Borer et al., 2007). The presence of exotic annuals increases the prevalence of virus infection and lowers the competitive ability of native grass species. Native perennial grasses dominate in the absence of disease because they outcompete annuals for light, nutrients and water, however when BYDV is present in the system exotic annual grasses are able to establish and coexist with the native perennials (Borer et al., 2007). Infection of B/CYDV in conjunction with environmental factors reduces plant fitness (Seabloom et al., 2009) ultimately altering competitive dynamics.

Across a landscape, natural grasslands may contribute to virus outbreaks in surrounding agricultural fields or they often harbor their own community of pests and pathogens distinct from the surrounding agricultural crops. Perennial grasses serve as a reservoir of virus inoculum for annual species and emerging agricultural crops. Our knowledge and understanding of insect vector movement between agricultural and natural landscapes is limited. Some studies suggest that viral species do not overlap between these two ecosystems, indicating distinct pathosystems

(Garrett et al., 2004), while others document overlap in the virus species present in agricultural and adjacent landscapes suggesting vector utilization of both habitats (Jones et al., 1990; Malmstrom, 1998). Furthermore, work in perennial mixed-species pastures has shown how plant age, community composition and vector populations can influence the identity and prevalence of viral species (Coutts and Jones 2002; Jones 2013) and is relevant to nonmanaged grasslands.

To better understand the ecology and epidemiology of B/CYDV among cropping systems, potential reservoir hosts need to be identified. Furthermore, to manage and conserve endangered grasslands a better understanding of the interactions among members of the plant community, including pathogens, is required. Therefore, the overall objective of this study was to examine nonmanaged grasslands in an agricultural matrix to gain a better understanding of the prevalence of agriculturally-important pathogens in habitats of conservation importance. We asked the following questions:

1. Are B/CYDV present in Palouse Prairie and conservation reserve program (CRP) habitats? If so, what are the prevalence, B/CYDV species present and potential aphid vectors in these habitats?
2. Is B/CYDV prevalence influenced by year, season, host type, site location and site type (CRP vs. prairie)?
3. Does host community composition vary among year, season, site location and site type (CRP vs. prairie)?
4. What is the importance of host community composition relative to the effect of year, season, site location, site type, and host type on B/CYDV prevalence?



5. Is B/CYDV prevalence in CRP and prairie habitats influenced by landscape factors, such as presence of cereal crops nearby?

6. Is B/CYDV prevalence altered by site-specific climate and soil variables?

## 2. Materials and Methods

### 2.1 STUDY AREA

The Palouse region is home to one of the most productive wheat-growing regions in the U.S. (Lister and Ranieri, 1995). Surveys were conducted in Palouse Prairie fragments located in southeastern Washington and adjacent northern Idaho (Fig. 1). Like many of the natural grasslands of the U.S., the Palouse Prairie is a highly fragmented landscape under pressure not only from a land-use perspective but also due to invasion by many introduced species (Noss et al., 1995; Lichtardt and Moseley, 1997; Looney and Eigenbrode, 2012; Ingwell and Bosque-Pérez, 2015a). Surveys were simultaneously carried out in conservation reserve program sites (Fig. 1). This program was established by the United States Department of Agriculture (USDA) Farm Service Agency in 1985 as a collaborative conservation effort coordinated among private land owners and intended to improve water quality, reduce soil erosion and increase undisturbed habitats for threatened or endangered species ([www.fsa.usda.gov](http://www.fsa.usda.gov)). Prairie remnants and CRP in the Palouse are the dominant habitats that contain grass species outside of agricultural fields; there are no managed pastures in the region and field margins tend to be weed free, likely as a result of management practices. We cannot rule the potential for B/CYDV and aphid reservoirs to occur at some locations along ditches and field margins but in general they are a minor component in the landscape and were not considered in this study.

### 2.2 DATA COLLECTION

Five Palouse Prairie remnants and six adjacent CRP habitats were selected based on availability, location and accessibility for this study (Fig. 1, Table 1). Each prairie site was paired with one adjacent CRP except for Smoot Hill. This location is unique because it sits on a large preserve maintained by Washington State University where weed and other management efforts are performed. Two CRP fields were selected at this site: a large CRP field planted with hard fescue (*Festuca brevipila* Tracey) located on the hillside above the prairie remnant and an additional CRP located farther from the prairie surrounded by conventionally managed agricultural fields, more similar to the other study sites. Each prairie and CRP site was sampled four times, fall 2010, spring 2011, fall 2011 and spring 2012, with the exception of Clyde prairie and CRP and the Kamiak Butte CRP sites, which were added to the study in spring 2011.

Each prairie and CRP was sampled along two parallel transects running perpendicular to the slope of each fragment. Transect length was adjusted according to the size of the site sampled. Every *Poaceae* species encountered within two meters of transect lines were identified and up to 15 samples per species, comprised of four leaves from each individual plant, were collected at each site and sampling time. Transects acted as a guide but did not restrict sampling. If a unique grass species was encountered when entering or exiting a site, samples were collected. Tissue collection was made at the time of flowering to aid in plant species identification. Aphids encountered during sampling were also collected and identified to species using available keys. Plant species were identified in the Lambert-Erickson Weed Herbarium and the Stillinger Herbarium at the University of Idaho. Grass and aphid samples were processed using reverse transcription polymerase chain reaction (RT-PCR) (see below) to determine viral infection. Cloning and sequence analysis (see below) were used to identify the viral species present in infected plant samples.

### 2.2.1 Nucleic acid extraction

For each plant sample, approximately 300-500 mg of tissue was ground in liquid nitrogen and total nucleic acid extraction performed using an adapted Dellaporta method (Pappu et al., 2005). Aphids were processed individually or three individuals from a single host plant pooled if more than one was present, and ground with a pestle directly in one mL of extraction buffer (100 mM Tris, pH 8.0, 50 mM EDTA, 500 nM NaCl and 10 mM mercaptoethanol). For plant samples, the buffer was added directly to the ground tissue after the liquid nitrogen evaporated. The extraction protocol followed Ingwell and Bosque-Pérez (2015a).

### 2.2.2 Reverse Transcription Polymerase Chain Reaction (RT PCR)

Reverse transcription (RT) reactions were done using M-MLV reverse transcriptase (Promega Corporation, Madison, WI, US) according to the manufacturer's instructions and as described by Ingwell and Bosque-Pérez (2015a). A unique set of primers designed by C. Lacroix was used to detect multiple species of B/CYDV. The forward primer (5'-CGGACARTGGTTTGTGG-3') and reverse primer (5'-TGGTAGGACTTRAGTAYTCC-3') were modified from previously published reports (Robertson et al., 1991; Chomič et al., 2010). BYDV-SGV, -RMV, -PAV, and -MAV infections produce a band 224 bp in size while CYDV-RPV produces a band 227 bp in size, which are visually indistinguishable on the agarose gel. The PCR protocol was performed according to Ingwell and Bosque-Pérez (2015a). The PCR products were viewed using gel electrophoresis following the procedure described in Ingwell et al. (2012; see Suppl. Mat.)

### 2.2.3 Cloning and sequence analysis

Thirty eight samples were selected for cloning and sequencing to confirm B/CYDV infection and determine viral species. Samples were selected to include a variety of sites, hosts and collection

times in order to examine geographic and seasonal variability as well as gauge the efficiency of the primers on a diverse set of host plant species. PCR products were purified on a low melting point (LMP) agarose gel with ethidium bromide staining using the Wizard® SV Gel and PCR Clean-Up System (Promega). Purified product was resuspended in 25-50 µL of nuclease-free water depending on the intensity of the band. The purified PCR product was cloned into pGEM® T-Easy vectors following the manufacturer's protocol (Promega) and transformed using *E.coli* cells DH5α. Clones were screened using EcoRI restriction enzymes following the manufacturer's protocol (Promega) by identifying plasmids containing inserts 224-227 bp in size. A minimum of three independent clones from each sample was sequenced in the forward direction by GENEWIZ, Inc. (South Plainfield, NJ, US) using the M13F (-21) primer. Sequence results were analyzed performing a nucleotide BLAST search on the National Center for Biotechnology's (NCBI) website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

#### 2.2.4 Landscape and Environmental Data

Land and climatic data for each sampling site was retrieved using the USDA Natural Resources Conservation Service (NRCS) Web Soil Survey (WSS, [websoilsurvey.sc.egov.usda.gov](http://websoilsurvey.sc.egov.usda.gov)). GPS coordinates for each site were used to locate the area of interest and sites delineated according to sampling. Land (i.e., soil type, soil series and composition) and climatic data (minimum and maximum mean annual precipitation and air temperature) were then exported per landform segment per site (Table 1). For sites with multiple landform segments (Kamiak Butte Prairie, Kramer CRP, Smoot Hill Prairie), site-level climatic variables were calculated based on the percentage of the site area covered by each segment.

Characteristics of each soil series present at the sampling sites (i.e., Tekoa, Palouse, Thatuna, Caldwell, Spokane, Libertybutte and Schumacher) (Table 1) were obtained from the USDA

Official Soil Series Description (OSSD, <https://soilseries.sc.egov.usda.gov>). From these descriptions, data were retrieved on soil pH for the horizons of each soil series for which the presence of at least a few roots was mentioned. Site-level soil pH was calculated based on the relative height of each horizon in a soil series, and on the percent of the site area constituted of each soil series. The taxonomic class of each soil series described in the OSSD was used to retrieve the associated drainage (Schaetzl et al., 2009) and productivity (Schaetzl et al., 2012) index from the USDA Forest Service website (<http://foresthealth.fs.usda.gov/soils>). Site-level drainage and productivity index were calculated based on the percent of the site area constituted by each soil series.

Cropland data were retrieved from the USDA-National Agricultural Statistics Service (NASS, 2010, 2011 and 2012) and were extracted using ArcGIS 10.0 (ESRI, Redlands, CA, USA) to quantify the proportion of cereal crops surrounding prairie and CRP patches in which sampling took place. A ring with a diameter of 1 km was drawn around each sampling point using the BUFFER tool. The area of the cropland data layer within the ring was retained using the CLIP tool. The area of each land cover type within the ring was extracted using TABULATE AREA on the resulting clipped layer. These values were added to a table using APPEND. This operation was repeated for each site and each year. This process was automated using the ModelBuilder in ArcGIS.

## 2.3 DATA ANALYSIS

All statistical analyses were performed using R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) (R CoreTeam, 2015). In all statistical analyses, especially for testing differences according to site type (i.e., CRP vs. prairie), a data set including samples from five pairs of sites was considered. Therefore, data from the Smoot Hill Lower CRP site were

used along with data from the local corresponding prairie site, and data from the atypical Smoot Hill Upper CRP site (i.e., *F. brevipila* field) were excluded.

First, differences were explored on virus prevalence (proportion of individuals infected) according to yearly sampling period (i.e., Fall 2010/Spring 2011 and Fall 2011/Spring 2012), season (i.e., fall vs. spring), site location and type (i.e., CRP vs. prairie), and plant host life history, origin and phylogenetic tribe. Mixed-effect generalized linear models were used (Pinheiro and Bates, 2000) in the lme4 R library with yearly sampling period, season, site location and type and host life history as random intercept terms within a logistic regression. The response variable was binomial, corresponding to the infection status (presence/absence of infection) in each sampled host.

Second, tests examined for variation in host community composition across yearly sampling periods, seasons, site location and type (i.e., CRP vs. prairie) using Permutational Multivariate Analysis of Variance (PerMANOVA) using the adonis function in the R vegan library (McCullagh and Nelder, 1989; Pinheiro and Bates, 2000). Host community composition was analyzed as a matrix that included the presence/absence of each host species at each site.

PerMANOVA is similar to MANOVA and redundancy analyses (Legendre and Anderson, 1999; Anderson, 2001; McArdle and Anderson, 2001) and constitutes an analysis of variance that compares distance matrices in which significance is determined using a permutation test. Here, 999 permutations and Jaccard's distance matrices were used. The model was tested with permutations left unconstrained and then with permutations constrained to occur within site pairs (i.e., location was treated as strata in the adonis function) for a robust test of differences in host community composition in CRP vs. prairie site within locations.

Third, the importance of several effects was assessed, including yearly sampling period, season, site location, site type, plant host identity and host life history, origin and tribe in accounting for variability in virus prevalence using a decision tree analysis (Breiman et al., 1984; Ripley, 2008) and the tree function in the R tree library. The tree is grown by binary recursive partitioning using the mean prevalence per species, site and sampling time as a response variable and choosing splits based on specified explanatory variables.

Finally, the effect of site-level land and climatic variables on mean virus prevalence was assessed. For this purpose, virus prevalence data were included for only the most widespread grass species (*P. spicata*, *F. idahoensis*, *Arrhenatherum elatius* L., *Alopecurus pratensis* L.) in order to reduce variability in mean site level virus prevalence due to host community composition. Mixed-effect generalized linear models were used (Pinheiro and Bates, 2000) in the lme4 R library with season and/or yearly sampling period as random intercept terms within a logistic regression. The effects of mean annual precipitation, mean annual air temperature, soil productivity index, soil drainage index, and soil pH were tested on mean site virus prevalence across years. The effect of the percent area covered with cereal crops (predominantly winter and spring wheat) within a 1-km radius around study sites was tested on mean site virus prevalence per year, and for each type of habitat (i.e., CRP vs. prairie).

### 3. Results

#### *3.1 Are B/CYDV present in Palouse Prairie and CRP habitats? If so, what are the prevalence, B/CYDV species present and potential aphid vectors in these habitats?*

A total of 2,271 plant samples were collected consisting of 30 species of grasses from the eleven prairie and CRP sites (Table 2). Among the samples, 46% tested positive for BYDV infection.

Virus infection was detected in 28 of the 30 different grass species collected (Table 2) with infection rates varying from 2 to 93% depending on species. In addition to the species listed here, the invasive grass *Ventenata dubia* (Leers) Cross. was also collected and found to be infected with BYDV (Ingwell and Bosque-Pérez, 2015a). We did not observe symptoms in any of the grass species encountered that were infected with BYDV. The virus species identified through the sequencing of a subset of 38 samples corresponded to BYDV-PAV and BYDV-SGV. While BYDV-SGV was the dominant species, identified at four prairie and four CRP sites, BYDV-PAV was detected at Kramer CRP, Paradise Ridge Prairie and both Smoot Hill CRP sites (Table 3). One mixed infection was detected at Clyde prairie in the perennial native *P. spicata* (bluebunch wheatgrass) in the fall of 2011 (Table 3).

Aphids were encountered only once during the entire sampling period. In July of 2012, aphids were found on five different plant samples at the Kamiak Butte CRP. *Sitobion* (formerly *Macrosiphum*) *avenae* were found on two samples of *Avena fatua* L. and two samples of volunteer wheat. *Metopolophium dirhodum* was found on one sample of *Aegilops cylindrica*. The *M. dirhodum* sample tested negative for B/CYDV infection while the host plant it was collected from tested positive for BYDV-SGV. One of the samples of *S. avenae* collected from *A. fatua* tested positive for B/CYDV and the other tested negative; neither of the sampled plants was infected. Both samples of *S. avenae* collected from volunteer wheat tested positive for B/CYDV while only one of the host plants was positive.

### 3.2 Is B/CYDV prevalence influenced by year, season, host type, site location and site type (CRP vs. prairie)?

BYDV prevalence varied significantly across years ( $p < 0.001$ , Fig. 2a) and seasons ( $p < 0.001$ , Fig. 2a) during which samples were collected. Mean BYDV prevalence increased from 33 %



during the first yearly period of sampling (Fall 2010/Spring 2011) to 61% during the second sampling period (Fall 2011/Spring 2012). Mean BYDV prevalence was also lower in spring than in fall, consistently across the two sampling periods (Fig. 2a).

BYDV prevalence differed significantly according to plant host life history, and was highest in perennial compared to annual hosts ( $p=0.02$ , Fig. 2b). Its prevalence was also highest among hosts belonging to the tribe Triticeae, followed by Poaceae and lowest among Bromaceae (Fig. 2c). These effects were robust after controlling for variation in virus prevalence across years, seasons, and site location and type. There was no difference in mean BYDV prevalence according to host origin (i.e., native vs. introduced,  $p=0.34$ ).

BYDV prevalence varied by site location (Fig. 3), with, in particular a higher prevalence at Smoot Hill relative to the other sites ( $p<0.001$ ). BYDV prevalence differed according to site type within each location. Mean BYDV prevalence in prairie patches compared to adjacent CRP lands was significantly higher at Kramer prairie ( $p<0.001$ ) and slightly but, non-significantly, higher at Kamiak Butte prairie ( $p=0.06$ ). In contrast, BYDV prevalence was significantly higher at CRP lands compared to adjacent prairie patches at Clyde prairie ( $p=0.03$ ) and Smoot Hill ( $p<0.001$ ). There was no difference in BYDV prevalence between the prairie and CRP patch at Paradise Ridge ( $p=0.4$ , Fig. 2d). The significant effects were robust after controlling for variation in BYDV prevalence across years, seasons and host type.

### *3.3 Does host community composition vary among year, season, site location and site type (CRP vs. prairie)?*

Plant community composition (i.e., presence/absence of grass species) was not statistically different ( $p=0.85$ ) across yearly period of sampling (i.e., Fall 2010/Spring 2011 vs. Fall

2011/Spring 2012). In contrast, the composition in plant species differed across seasons ( $p < 0.001$ ), site location ( $p < 0.001$ ) and site type (CRP vs. prairie,  $p < 0.001$ ) in permanova tests in which permutations were left unconstrained. The season ( $p < 0.001$ ) and site type ( $p < 0.001$ ) effects remained significant when permutations were constrained to occur within locations.

### 3.4 *What is the importance of host community composition relative to the effect of year, season, site location, site type, and host type on BYDV prevalence?*

The decision tree analysis identified the yearly period of sampling as the first main variable that accounted for most of the variability in the mean BYDV prevalence per species, site and sampling time. The analysis thus split the data set into two subsets of samples that displayed the greatest differences in mean prevalence (i.e., 30% in Fall 2010/Spring 2011 and 65% in Fall 2011/Spring 2012).

For each of these subsets, plant host identity was then determined as the second variable that accounted for most of the variability in mean BYDV prevalence. This resulted in binary partitioning of each of these subsets (i.e., Fall 2010/Spring 2011 and Fall 2011/Spring 2012) into two other datasets that displayed the greatest differences in mean prevalence (i.e., 20% and 48%; and of 42% and 71%, respectively). For each of these two splits, the samples of five plant host species were consistently associated with the datasets showing the highest BYDV prevalence. These five species included the perennial introduced *A. elatius* and *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, and the perennial US natives *Koeleria macrantha* (Ledeb.) Schult., *Poa pratensis* L., and *P. spicata*. These species were among the hosts that displayed the highest mean BYDV prevalence across years, seasons and sites (Fig. 4). The further binary partitioning of these data sets resulted in subsets with greatest differences in mean BYDV prevalence according to season, site location and host identity.

### 3.5 Is BYDV prevalence in CRP and prairie sites influenced by landscape factors such as presence of cereal crops nearby?

We found a correlation between the percent area covered with cereal crops within a 1-km radius around CRP and prairie sites studied and mean BYDV prevalence. As the proportion of grain (mostly wheat) in the landscape increased, so did the prevalence of BYDV among grasses in the nonmanaged habitats examined (Fig. 5). The correlation between mean percent area covered with cereal crop within a 1-km radius and mean BYDV prevalence at CRP sites was significant ( $p < 0.001$ ) only when one outlier data point (Smooth Hill CRP site in 2010,  $x = 0.01$ ,  $y = 0.93$ , Fig. 5) was removed. The correlation between mean percent area covered with cereal crop within a 1-km radius and mean BYDV prevalence at prairie sites was significant with ( $p < 0.001$ ) and without ( $p < 0.001$ ) one outlier data point (Kamiak Butte prairie site in 2010,  $x = 0.28$ ,  $y = 0.13$ , Fig. 5). This effect was robust after controlling for variability in mean BYDV prevalence across yearly sampling periods and seasons.

### 3.6 Is BYDV prevalence altered by site-specific climate and soil variables?

The among site variability in mean soil productivity index, soil drainage index, range in annual air temperatures and range in annual precipitation was insufficient to make any robust inference on a potential correlation with virus prevalence (Supplemental Fig. S1). Nevertheless, there was a positive correlation between site level soil pH and mean BYDV prevalence across years and seasons ( $p < 0.001$ , Supplemental Fig. S1).

## 4. Discussion

This is the first comprehensive sampling of *Poaceae* species in Palouse Prairie and CRP habitats documenting infection of BYDV among a large variety of plant species (Table 2). The presence

of BYDV in prairie and CRP habitats has the potential to impact conservation and restoration efforts for the Palouse Prairie as well as disease dynamics in agricultural fields. More broadly, these results highlight the importance of understanding disease dynamics among fragmented habitats within an agricultural matrix in order to better manage plant viruses at a landscape scale, an interface that has more recently drawn attention (Vincent et al., 2014).

We have shown that BYDV is prevalent in both native (i.e., *F. idahoensis*, *P. pratensis*, *P. spicata*) and exotic grass species, which include cheatgrass (*Bromus tectorum*) and the newest invader, *Ventanata dubia* (Ingwell and Bosque-Pérez, 2015a) among the sites sampled. There was significant variation in virus prevalence depending on the season when sites were surveyed (fall vs. spring) as well as between years (Fig. 2a). Additionally, BYDV was shown to be more prevalent among the perennial species sampled compared to annual species (Fig. 2b). This might be the result of symptomless tolerant perennial plants serving as reservoirs of the pathogen each year. In addition, perennials are likely to be accumulating more BYDV infection over time because they live longer than annuals (which emerge BYDV-free each year) and thus experience a higher probability of becoming infected over time. Lastly, five grass species were identified that were associated with the highest virus prevalence (Fig. 4), all of which are perennial species. Taken together, these results show that perennial species are an important aspect of plant pathogen dynamics in these habitats.

Our results further show that BYDV prevalence correlates to host community composition. The driver of this correlation could be changes in the abundance of particular host plants, which we did not measure in this study, especially the five most associated with high BYDV prevalence (Fig. 4). Understanding host abundance would help elucidate the variability among seasons and years documented here. Alternatively, differences in BYDV prevalence might be related to plant

host traits including the ability of a plant to become infected by BYDV, the severity of symptoms that develop, or the attractiveness to vectors. Other factors such as the age of the prairie remnant or CRP or management practices are likely to influence the incidence of virus, especially among the perennial species. Work in perennial pastures in Australia has shown that BYDV incidence increases with pasture age and that mowing can change the incidence, perhaps as a result of disrupting competition between infected and noninfected individuals (Coutts and Jones 2002). Regardless of the mechanism, avoiding grass species that become infected with BYDV as demonstrated here and elsewhere (see Ingwell and Bosque-Pérez, 2015b), by excluding them from CRP seed mixes, may help lower BYDV inoculum in the landscape and has been suggested as a means to reduce incidence of B/CYDV in perennial pastures (Jones 2013).

The differences in virus prevalence documented among individual species across the Palouse landscape are relevant to previous work in a California grassland system where BYDV is present and has been shown to alter community composition. The invasive species *B. tectorum* under BYDV-free conditions, was unable to persist among the competitively-dominant native species without some sort of perturbation or land-use change (Seabloom et al., 2003; Corbin and D'Antonio, 2004). Work examining the effects of BYDV-infection on the competitive interactions among native and exotic species in this community has demonstrated the crucial role that plant viruses play in shaping plant community structure (Borer et al., 2007). Efforts to restore native grasslands need to consider these elusive factors. Our results documenting high prevalence of BYDV among perennial species provide evidence in support of the disease facilitation hypothesis which proposes that viral infection facilitated the invasion of Eurasian grasses that displaced native species in California, USA (Malmstrom et al., 2005a). Additional research is required to examine the impacts of competition among native grasses, and invaders

such as *B. tectorum* and *V. dubia* in Palouse prairie habitats and how BYDV prevalence among these hosts could mediate their interactions.

Knowledge of vector competence and viral species can also elucidate viral dynamics. Given that very few aphids were encountered and these occurred in only one of the CRP sites and none of the prairie remnants, the BYDV infection that we have documented is most likely due to non-colonizing aphid species. These are aphids using the grasses as hosts for a period of time long enough for virus transmission to occur (Power et al., 1991). This was verified in the laboratory for *V. dubia* (Ingwell and Bosque-Pérez, 2015a). In addition, root aphids may be contributing to viral dynamics (Paliwal, 1980; Jedlinski, 1981), but were not sampled in this study. The two aphid species observed, *S. avenae* and *M. dirhodum*, are both competent vectors and could be contributing to BYDV-PAV infection; the dynamics of BYDV-SGV are most likely attributable to *S. avenae* or other aphids not encountered as *M. dirhodum* transmission of this viral species has never been documented (Bencharki et al., 2000; Gray and Gildow, 2003).

The infection level of 40% among annual grasses indicates that there were migrant aphid vectors visiting these habitats on a regular basis contributing to BYDV spread (Table 3, Fig. 4). The sources of inoculum for these infections could have arisen from infected perennial hosts within the CRP or prairie, or from neighboring cereal crops. Exploring the viral and vector species found among annual hosts, including wheat fields, could lend insight to yearly variations in vector composition and abundance in the community and interactions between agricultural and nonmanaged habitats. Furthermore, a better understanding of vector preferences of grass hosts in prairie remnants would provide critical information for management practices and enhance prairie conservation efforts.

Agriculture, being the dominant land-use in the region, ultimately plays an important role in disease dynamics across the landscape. One important result stemming from this work is the relationship between cereal grain cover and prevalence of BYDV infection in nonmanaged habitats (Fig. 5). While progress is being made, there are still many discrepancies between the B/CYDV species being identified in nonmanaged grasses and those causing disease outbreaks in cereal crops. For instance, in Idaho, five species of B/CYDV (RPV, MAV, PAV, RMV, SGV) have been detected (Forster, 1983). However, historically the most consistently identified species among wheat crops in southeastern Idaho has been SGV linked with the vector *Schizaphis graminum* (Forster et al., 1990). In the western regions of Idaho and Washington state, PAV and RPV have historically been the dominant viral species found in cereal crops and viral disease outbreaks have been associated with large populations of *R. padi* and *M. dirhodum* (Halbert and Pike, 1985; Gildow, 1990). This information is more than 20 years old, therefore updated information on B/CYDV incidence in wheat fields may help elucidate vector utilization among these habitat types. The most recent survey of cereal aphids in wheat conducted in and around our study region in 2011-2013 found *S. avenae* and *Metopolophium festucae cerealium* to be the most abundant aphid species (Halbert et al., 2013). *S. avenae* is an efficient vector of BYDV-MAV but also has been shown to transmit BYDV-PAV (Rochow, 1969). *M. festucae cerealium* was reported for the first time in the U.S. only recently (Halbert et al., 2013) and does not appear to play a role in BYDV epidemics in the region as laboratory experiments demonstrated its inability to transmit BYDV (Sadeghi et al., 2016).

Among the current study sites located in north central Idaho and adjacent eastern Washington, PAV and SGV were identified as the prevalent B/CYDV species in nonmanaged grasslands. However, since only a small portion of the plant samples were subjected to cloning and

sequencing in the current study, it is not possible to exclude the possibility that other B/CYDV species are present in the region, but went undetected. Regardless, results from these samplings, and data from aphid collections in wheat fields, suggest that *S. avenae* could be utilizing both habitats and transmitting both PAV and SGV between habitats. Simultaneous investigations of vector and host infection status in agricultural and adjacent nonmanaged habitats will help clarify the interactions between the two. A better understanding of these interactions can provide crucial information for improving epidemiological models (such as BYDV PREDICTOR; see Thackray et al., 2009).

## 5. Conclusions

Apparent from the results presented here is the potential role that nonmanaged habitats play in agricultural viral pathogen epidemics and vice versa. BYDV was prevalent in both prairie and CRP habitats and infection rates were high at some of the sites. Important variables influencing BYDV prevalence were identified and include the time of sampling, host identity and proportion of grain cover crop within a 1-km radius. These results draw attention to the fact that as conservationists attempt to restore grasslands, viral pathogens often remain unapparent or overlooked and have the potential to impact restoration success (Cooper and Jones, 2006). In order to better understand the dynamics of viral pathogens in agricultural and nonmanaged habitats experiments need to be designed to specifically explore the seasonal dynamics and interactions between these habitats and their viral pathogens and vectors.

The relationship between cereal grain cover and BYDV prevalence in nonmanaged habitats highlights the importance of considering the landscape when working to conserve a fragmented habitat, such as the Palouse prairie. The location and proportion of wheat grown in the region has an impact on the prevalence of BYDV in this endangered ecosystem. One recommendation



stemming from this work would be to buffer endangered habitats with non-host crops, in order to reduce pathogen transmission between agricultural and nonmanaged habitats.

Another recommendation is to consider the susceptibility of species selected for conservation efforts, such as the CRP lands. Selecting plant species that help achieve the overall goal of reducing run-off and sedimentation while also decreasing viral pathogen reservoirs could be achieved by choosing *Poaceae* species with low BYDV prevalence (Fig. 2c and Fig. 4) or those that are not hosts to it. Comprehensive lists of grass species and B/CYDV susceptibility or detection can be found in D'Arcy, 1995, Malmstrom, 1998, Jones, 2013, and Ingwell and Bosque-Pérez, 2015b. In some situations the natural host range of agriculturally-important pathogens may not be fully known, but efforts to explore this prior to selecting or recommending seed mixes have important implications for disease management and conservation of endangered habitats.

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## FIGURE LEGENDS

**Figure 1** Map of locations at which samples were collected. 1, Clyde Prairie; 2, Clyde CRP; 3. Kamiak Butte Prairie; 4. Kamiak Butte CRP; 5. Paradise Ridge Prairie; 6. Paradise Ridge CRP; 7. Kramer Prairie; 8. Kramer CRP; 9. Smoot Hill Prairie; 10. Smoot Hill Upper CRP; 11. Smoot Hill Lower CRP.

**Figure 2** Virus prevalence (percentages of individuals infected) across years and seasons during which samples were collected (A), between annual (A) and perennial (P) grass hosts (B), and among the three major tribes of grasses collected (C). Virus prevalence varied between prairie and CRP patches among the sites sampled (D; PR = Paradise Ridge, KB = Kamiak Butte, SH = Smoot Hill). Error bars represent  $\pm 1$  SEM.

**Figure 3** A map illustrating the prevalence of virus infection at each location averaged over all sampling times.

**Figure 4** Mean virus prevalence (percentages of individuals infected) per host species across years, seasons and sites. Full names corresponding to species abbreviations are shown in Table 2.

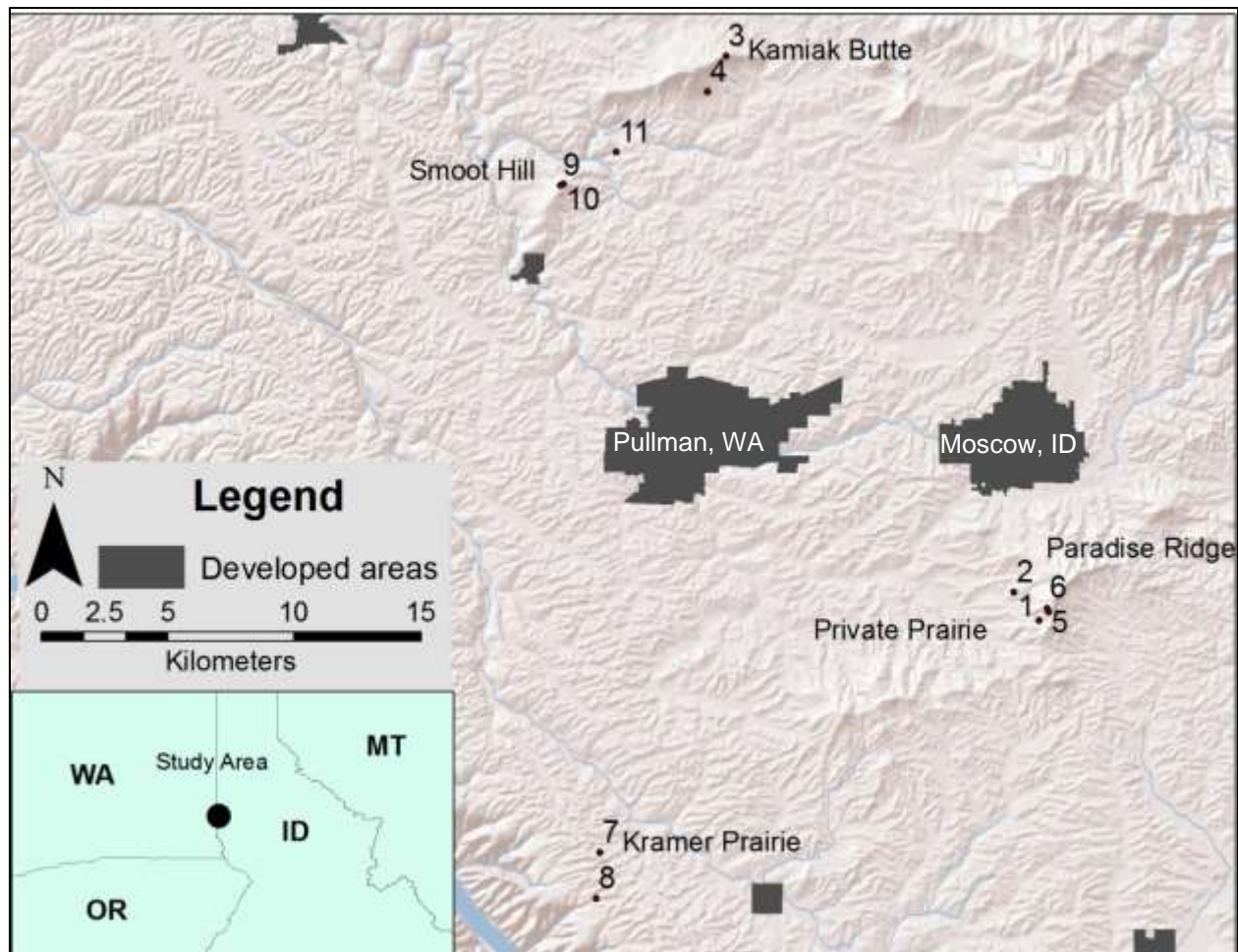
\* Five species (*Arrhenatherum elatius*, *Thinopyrum intermedium*, *Koeleria macrantha*, *Poa pratensis* and *Pseudoroegneria spicata*) were identified through a decision tree analysis as hosts most likely to be associated with high virus prevalence. Error bars represent  $\pm 1$  SEM.

**Figure 5** The prevalence of BYDV infection in nonmanaged habitats increases as the percentage of grain (winter and spring wheat) increases within a 1-km radius around each site. The prevalence data was calculated based on the most widespread grass species (*P. spicata*, *F. idahoensis*, *A. elatius*, and *A. pratensis*). The solid and dotted lines show the slope of the

significant relationship between the percentage of cereal crop in the landscape and virus prevalence at CRP and prairie sites, respectively.

## FIGURES

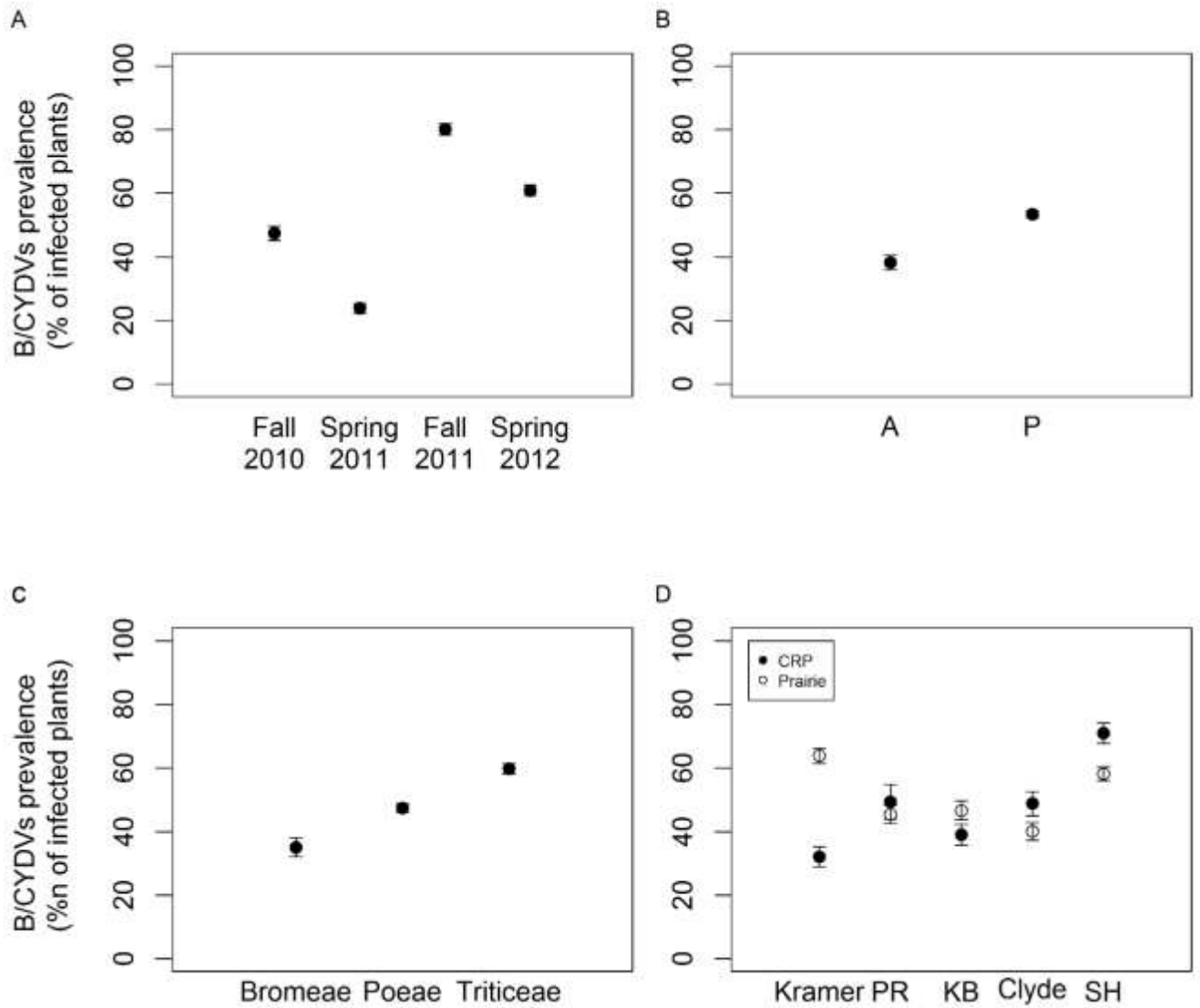
Figure 1



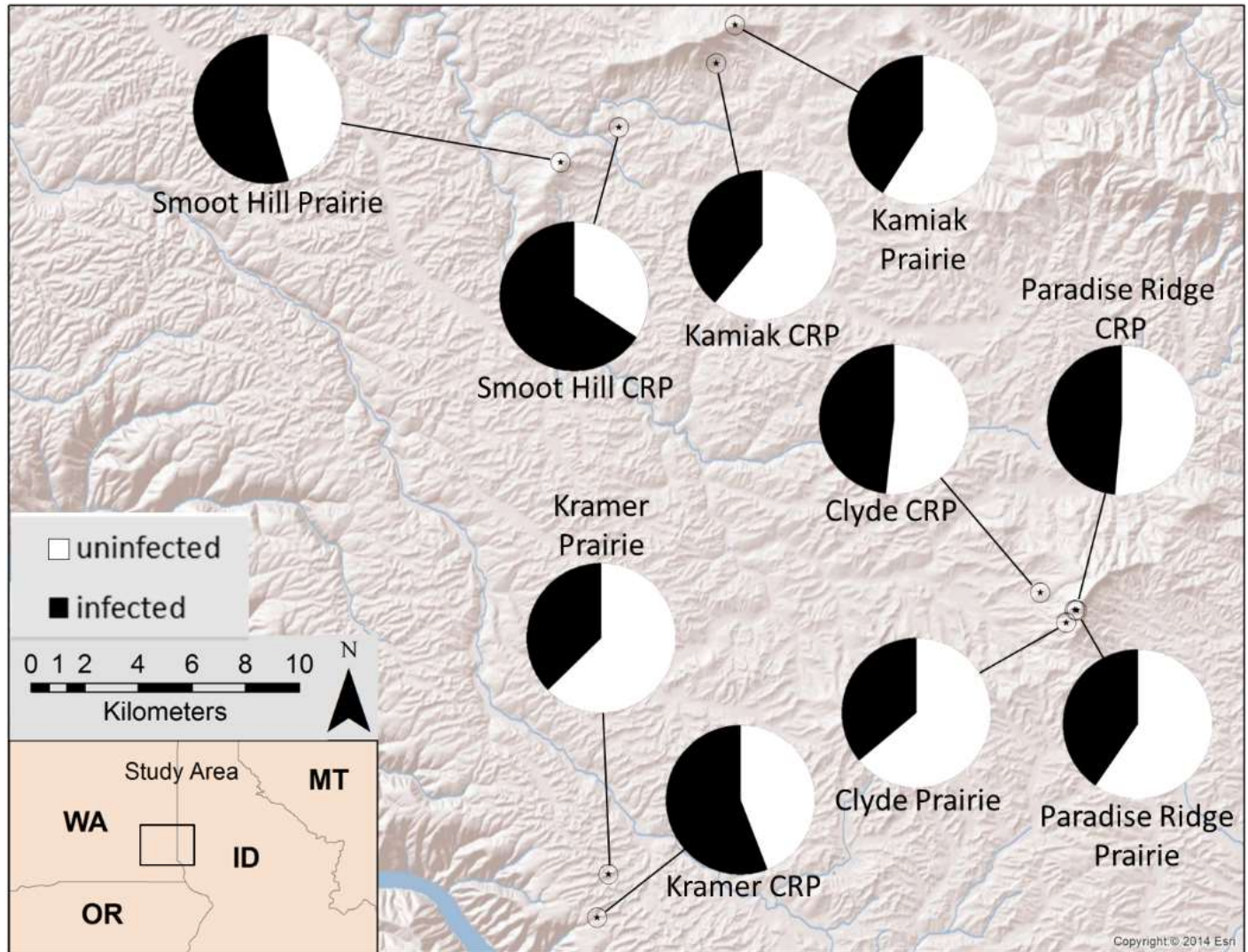
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Figure 2

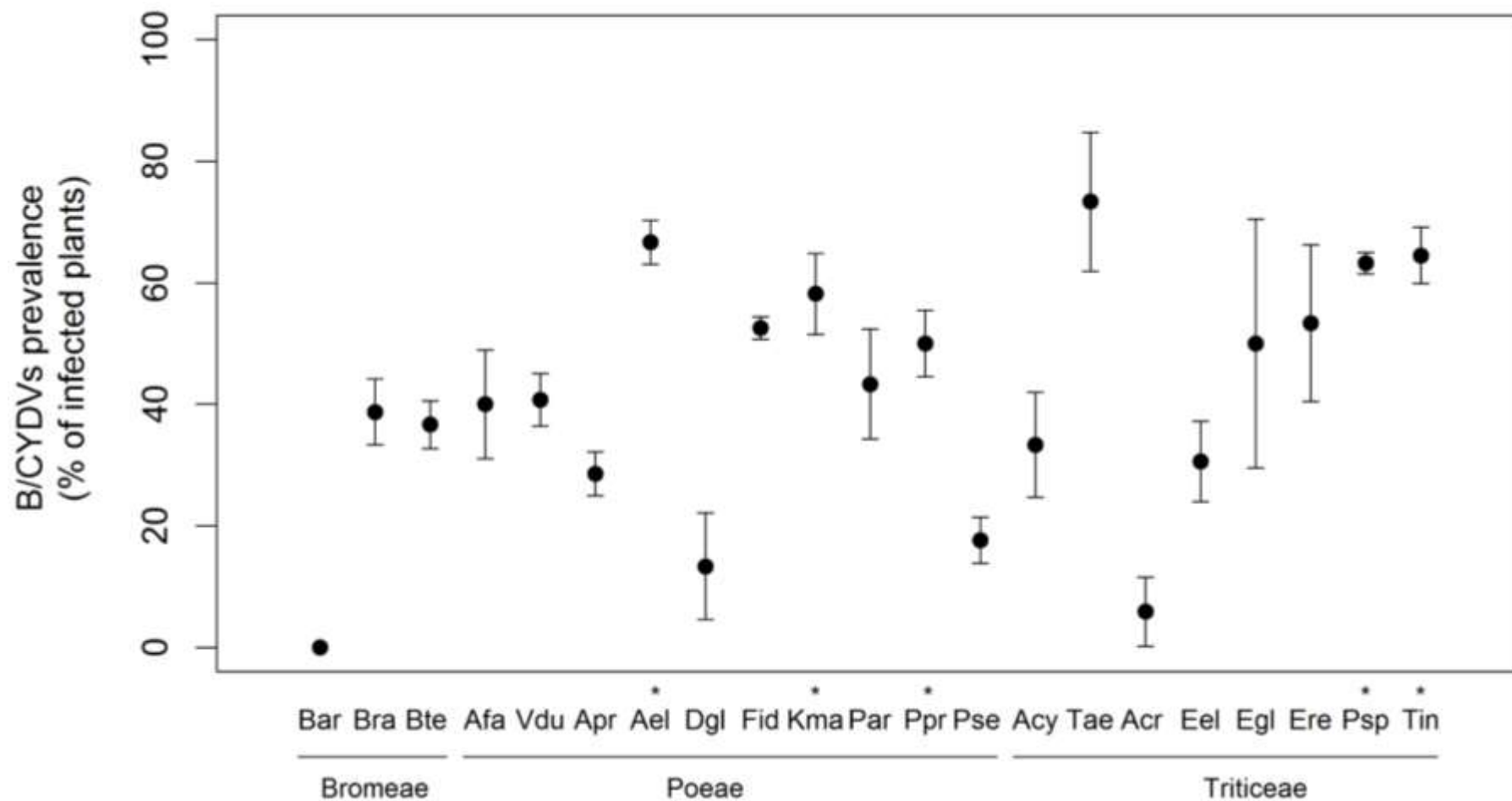


**Figure 3**





1 **Figure 4**

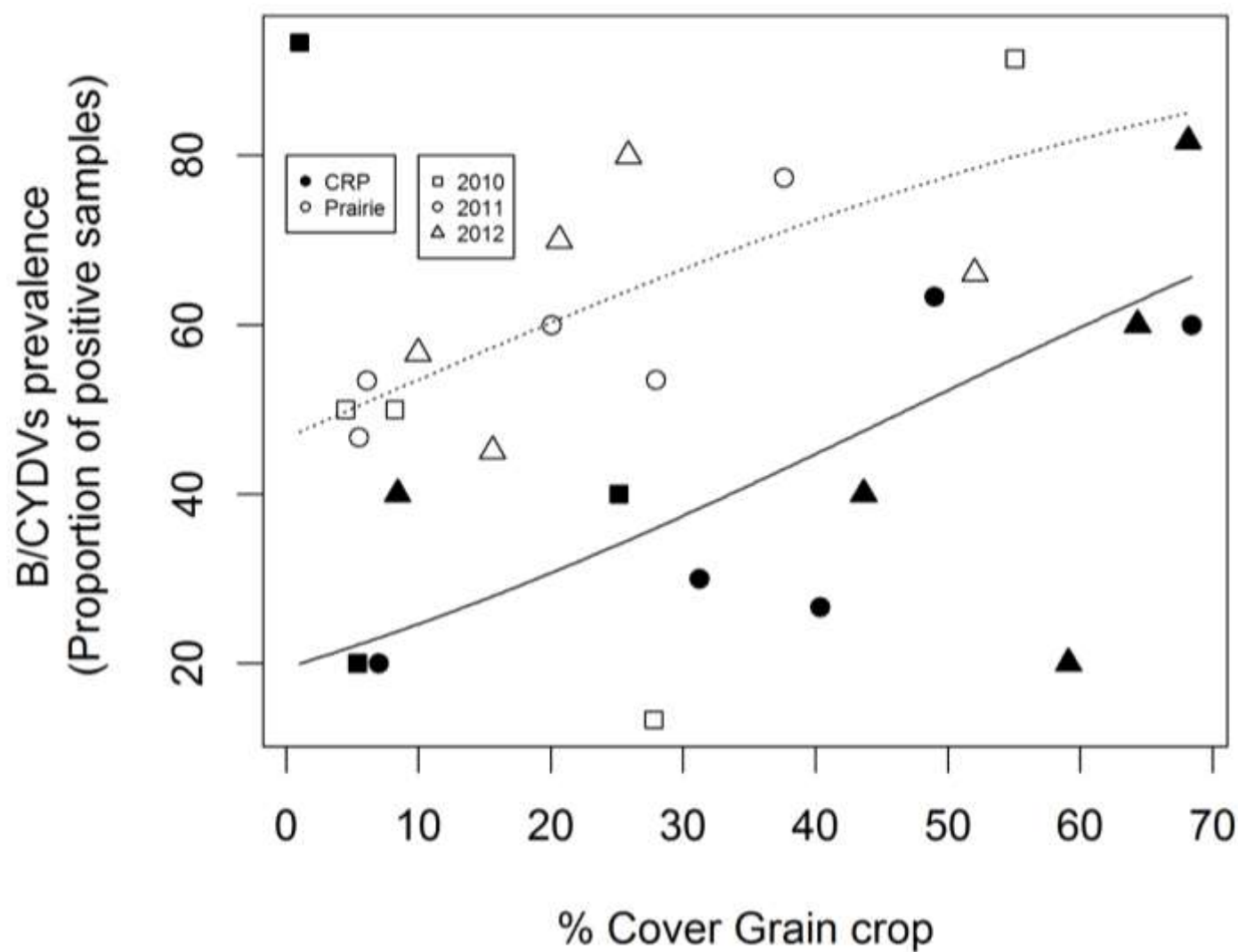


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3 **Fig. 5**



4



# 5 TABLES

6 **Table 1.** Site location and characteristics retrieved from the USDA-Natural Resources Conservation Service (USDA-NRCS) Web Soil  
7 Survey tool [Accessed April 2014] and cropland data from USDA National Agricultural Statistics Service (USDA-NASS).

Site	Location	Latitude, longitude	Ownership	Soil Type	% Compositi on	Mean soil drainage index	Mean soil productiv ity index	Mea n soil pH	Mean range ppt (inches)	Mean range air temp (F)	% Cereal crop in 1km <sup>2</sup> radius			
											2010 Fall	2011 Sprin g	201 1 Fall	2012 Sprin g
Clyde Prairie	Latah, Co., ID	N 46° 40.007' W 116° 59.291'	Private	Spokane ashy loam	100.00	28.00	15.00	6.35	6.00	4.00	NA	0.05	0.0 5	0.16
Clyde CRP	Latah, Co., ID	N 46° 40.612' W 117° 00.051'	Private	Palouse silt loam	100.00	30.00	16.00	6.87	10.00	4.00	NA	0.49	0.4 9	0.59
Kamiak Butte Prairie	Whitman Co., WA	N 46° 52.055' W 117° 09.023'	Whitman Co. Parks/Priva te	Tekoa silt loam	72.50	31.00	16.00	6.34	7.25	2.18	0.28	0.38	0.3 8	0.26
				Tekoa stony silt loam	27.50									
Kamiak Butte CRP	Whitman Co., WA	N 46° 51.274' W 117° 09.576'	Private	Palouse silt loam	100.00	30.00	16.00	6.87	6.00	4.00	NA	0.31	0.3 1	0.64
Paradise Ridge Prairie	Latah, Co., ID	N 46° 40.266' W 116° 59.032'	Private	Libertybutte- Schumacher complex	100.00	26.82	13.06	6.68	6.00	4.00	0.05	0.06	0.0 6	0.10
Paradise Ridge CRP	Latah, Co., ID	N 46° 40.261' W 116° 58.978'	Private	Spokane ashy loam	100.00	28.00	15.00	6.35	6.00	4.00	0.05	0.07	0.0 7	0.08
Kramer Prairie	Whitman Co., WA	N 46° 34.926' W 117° 12.680'	Washington State Univ.	Palouse-Thatuna silt loams	100.00	29.53	16.00	6.72	6.00	5.00	0.55	0.28	0.2 8	0.52
Kramer CRP	Whitman Co., WA	N 46° 34.050' W 117° 13.008'	Private	Caldwell silt loam, drained	60.50	43.93	16.00	7.01	6.00	3.51	0.25	0.40	0.4 0	0.44
				Palouse silt loam	27.50									
				Palouse-Thatuna silt loams	12.10									
Smoot Hill Prairie	Whitman Co., WA	N 46° 49.276' W 117° 14.143'	Washington State Univ.	Palouse silt loam	76.10	30.24	16.00	6.75	5.48	3.32	0.08	0.20	0.2 0	0.21

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				Tekoa silt loam	9.10									
				Tekoa stony silt loam	14.80									
Smoot Hill CRP	Whitman Co., WA	N 46° 49.986' W 117° 12.428'	Washington State Univ.	Palouse silt loam	100.00	30.00	16.00	6.87	6.00	4.00	0.01	0.68	0.68	0.68

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**Table 2** A comprehensive list of all species collected at Palouse Prairie and CRP sites. Species abbreviations relate to Figure 4.

Plant Species	Tribe	Growth habit <sup>a</sup>	Native status <sup>b</sup>	Proportion infected with BYDV	Host in current study
<i>Aegilops cylindrical</i> (Acy)	Triticeae	A	I	10/30 (33%)	Y
<i>Agropyron cristatum</i> (Acr)	Hordeae	P	I	17/62 (27%)	Y
<i>Alopecurus pratensis</i> (Apr)	Aveneae	P	I	44/154 (29%)	Y
<i>Arrhenatherum elatius</i> (Ael)	Poeae	P	I	123/185 (66%)	Y
<i>Avena fatua</i> (Afa)	Aveneae	A	I	12/30 (40%)	Y
<i>Bromus inermis</i>	Bromeae	P	I,N	66/180 (37%)	Y
<i>Bromus arvensis</i> (Bar)	Bromeae	A	I	0/15 (0%)	N
<i>Bromus racemosus</i> (Bra)	Bromeae	A	I	27/65 (42%)	Y
<i>Bromus tectorum</i> (Bte)	Bromeae	A	I	55/150 (37%)	Y
<i>Dactylis glomerata</i> (Dgl)	Poeae	P	I	9/30 (30%)	Y
<i>Elymus elymoides</i> (Eel)	Triticeae	P	N	15/49 (31%)	Y
<i>Elymus glaucus</i> (Egl)	Triticeae	P	N	3/6 (50%)	Y
<i>Elymus repens</i> (Ere)	Triticeae	P	I	8/15 (53%)	Y
<i>Elymus</i> sp.	Triticeae	ND	ND	17/30 (57%)	Y
<i>Festuca brevipila</i>	Poeae	P	I	27/60 (45%)	Y
<i>Festuca idahoensis</i> (Fid)	Poeae	P	N	152/293 (52%)	Y
<i>Festuca</i> sp.	Poeae	ND	ND	0/1 (0%)	N
<i>Koeleria macrantha</i> (Kma)	Aveneae	P	N	23/40 (58%)	Y
<i>Lolium perenne</i>	Triticeae	P/A	I	2/6 (33%)	Y
<i>Phalaris arundinacea</i> (Par)	Poeae	P	N	25/45 (56%)	Y
<i>Poa bulbosa</i>	Poeae	P	I	11/51 (22%)	Y

				42/84	
<i>Poa pratensis</i> (Ppr)	Poeae	P	N	(50%)	Y
<i>Poa secunda</i> (Pse)	Poeae	P	N	1/42 (2%)	Y
				17/60	
<i>Poa secunda</i> var. <i>ampla</i>	Poeae	P	N	(28%)	Y
				10/15	
<i>Poa</i> sp.	Poeae	ND	ND	(67%)	Y
				213/348	
<i>Pseudoroegneria spicata</i> (Psp)	Triticeae	P	N	(61%)	Y
				83/122	
<i>Thinopyrum intermedium</i> (Tin)	Triticeae	P	I	(68%)	Y
				18/30	
<i>Triticum aestivum</i> (Tae)	Triticeae	A	I	(60%)	Y
				59/145	
<i>Ventenata dubia</i> (Vdu)	Poeae	A	I	(41%)	Y
				11/15	
Morphospecies 1		P		(73%)	Y
				23/59	
Morphospecies 2		P		(39%)	Y
				1123/241	
Total				7 (46%)	

<sup>a</sup> A = annual, P = perennial, ND = not determined

<sup>b</sup> I = introduced, N = native, according to USDA Plants Database for the lower 48 United States, ND = not determined

**Table 3** Characteristics of selected plant samples and identity of virus species.

Plant host <sup>a</sup>	Location <sup>b</sup>	Collection time	Virus species identified
<i>Aegilops cylindrica</i> (A)	Kamiak Butte CRP	July 2012	SGV
<i>Arrhenatherum elatius</i> (P)	SH Lower CRP	October 2010	SGV
	SH Lower CRP	June 2011	PAV
	SH Lower CRP	October 2011	SGV
	SH Lower CRP	July 2012	SGV
<i>Bromus inermis</i> (P)	SH Upper CRP	October 2010	SGV
	SH Upper CRP	October 2011	SGV
	SH Upper CRP	July 2012	PAV
	Kramer Prairie	May 2012	SGV
<i>Bromus tectorum</i> (A)	Kramer CRP	October 2010	PAV
	Kramer CRP	May 2012	SGV
<i>Elymus glaucus</i> (P)	Clyde Prairie	July 2012	SGV
		September 2010	SGV
<i>Festuca idahoensis</i> (P)	Paradise Ridge Prairie	June 2011	PAV
	Paradise Ridge Prairie	November 2011	SGV
	Paradise Ridge Prairie	May 2012	SGV
	SH Prairie	July 2012	SGV
<i>Pseudoregneria</i> sp. (P)	Kramer CRP	October 2010	SGV
		November 2011	PAV/SGV mix
<i>Pseudoregneria spicata</i> (P)	Clyde Prairie	July 2012	SGV

<sup>a</sup> A = annual, P = perennial<sup>b</sup> SH = Smoot Hill