PROJECT NO: BJKT49, BJKT50, BJKT51, BJKT52

TITLE: The Importance of Corn and Grassy Weeds in BYDV Spread

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JUSTIFICATION: Barley yellow dwarf virus (BYDV) is an agronomically important disease of cereal crops worldwide. Apart from infecting crops such as barley, oats and wheat, corn and several species of other grasses may also serve as hosts and potentially function as important virus reservoirs (Hadi 2009). Among several species of cereal aphids, the bird cherry-oat aphid is an efficient vector of BYDV. BYDV outbreak in 2012-2013 (Marshall and Rashed, 2014) and in 2014-2016 in south-central Idaho has been attributed to BYDV-PAV species, which is predominant in Idaho.

There has been a recent increase in corn acreage in central and eastern Idaho. Moreover, our preliminary results, from the first two years of our survey, indicated that some species of grassy weeds may be source of BYDV, supporting those of previous findings indicating that BYDV is prevalent in grassy weeds in northern Idaho pastures (Ingwell and Bosque-Perez, 2015). Also, there is a report of wild annual and perennial grasses, volunteer cereals and even neighboring cultivated grain crops acting as alternative hosts for the virus in the absence of cultivated commodities (Hadi 2009). Limited information is available on corn and grassy weeds as a host to pathogens and insects in south-central Idaho. The potential of corn and grassy weeds to host BYDV may also have implications in the virus transmission from corn and weeds to winter wheat. An understanding of the BYDV infection of corn and grassy weeds and the ability of predominant vectors to acquire and then transmit the virus to the cultivated hosts will help to improve our understanding of regional BYDV epidemiology, and will assist with decision making process and management efforts.

We will continue to evaluate the following hypothesis and the related three objectives.

Hypothesis: Increased corn production and presence of local wild grasses impact BYDV epidemiology/spread in central and eastern Idaho. To evaluate this hypothesis we will address the following **Objectives:**

I. Continue an area-wide sampling of wild grassy species from fields located in central, eastern and northern (expansion) Idaho to identify potential BYDV hosts,

II. Determine cereal aphid species and their movement pattern from potential BYDV reservoirs to winter wheat,

III. Examine the possibility of BYDV transmission to grassy weeds and evaluate the efficiency of transmission from corn and grassy weeds, in particular those that were found to host BYDV (Obj. 1), to winter wheat.

PROCEDURES: To address **Objective I,** we will continue plant sampling within, and near, ~100 (an expansion from the last two years) field sites in central, eastern and northern Idaho that were previously tested positive for BYDV. Collected samples will be tested for BYDV using ELISA. In **Objective II,** aphid movement will be monitored using yellow sticky traps, and yellow pan traps, in several corn fields and winter wheat fields near to corn throughout central and eastern Idaho from July to November. Yellow sticky traps will be set up on the edges of selected corn fields (N = 3) and within winter wheat facing the corn (N = 6). Traps will be replaced every 3-4 weeks with new ones to monitor aphid movement from corn to winter wheat. Cereal aphids will be counted from collected yellow sticky traps and identified in our yellow pan traps. We expect that mean aphid numbers start to increase in emerging winter wheat fields as corn starts to mature. Leaf samples from corn, grassy weeds and winter wheat will be sampled from plants surrounding sticky traps to test for BYDV infection. As BYDV presence would not necessarily be indicative of the host plant role in pathogen spread, **Objective III** is set to study BYDV transmission efficiency from corn and potential grassy weed hosts (selected from those that tested positive for BYDV in the field) to wheat host. Ten viruliferous aphids from our BYDV infected colony will be given 5 day-inoculation access period (IAP) on corn and grassy weeds, two weeks after emergence. Aphids will be mechanically removed when the IAP elapsed. Inoculated plants will be maintained inside cages for three weeks. After three weeks, about 10 clean aphids will be placed on inoculated corn and grassy weeds for a 5-day acquisition access period (AAP). Then they will be transferred to newly emerged winter wheat (WB-Junction) for a 5-day IAP to quantify transmission success. Corn, grassy weeds and winter wheat will be tested for BYDV infection and BYDV titer will be quantified using qPCR, 30 days post inoculation. QPCR results will give us information regarding BYDV infection level in corn and grassy weeds, which would allow examining whether transmission success is influenced by BYDV quantity at the source plant.

DURATION: This would be the 3rd year of a 3-year proposal.

COOPERATION: Proposed research is being carried out collaboratively among Dr. Rashed, Dr. Hutchinson, Dr. Marshall and Dr. Bosque-Pérez's programs in cooperation with several regional cereal growers.

ANTICIPATED BENEFITS/EXPECTED OUTCOMES/INFORMATION TRANSFER:

This research in the past two years has resulted in the finding of natural BYDV infection in foxtail barley, for the first time, in Idaho. The ability of BYDV-PAV to infect foxtail barley, green foxtail, downy brome and corn in the greenhouse and transmission from infected grassy weeds and corn to winter wheat were demonstrated. Thus, the above-mentioned grasses and corn are hosts to BYDV and may serve as a source of virus inoculum. These findings have important implications in BYDV ecology/epidemiology in areas where the evaluated species are present. Based on our 2014-2016 efforts and results from field survey and greenhouse, we are expanding this research for the third year to monitor more fields to identify key aphid vectors and patterns of their movement from corn to nearby winter wheat. Additional grassy weed species will be tested for BYDV presence. Information is expected to help growers in BYDV management (i.e. applying seed treatments and managing cereal aphids in corn and/or other host plants). The three-year data generated through our research will provide us with sufficient information to be communicated to growers and the scientific community through cereal schools, one CIS publication, and conferences and, at least, one referred journal publication.

LITERATURE REVIEW:

Barley yellow dwarf (BYD) is one of the most widespread and damaging viral diseases of grasses and cereal crops worldwide (D'Arcy, 1995). Corn and some other grass species may also function as important virus reservoirs (Hadi 2009). Previous studies in California, and Idaho identified several weed species as potential natural hosts and reservoirs of BYDV. Also, it has been shown that *Avena fatua* and several other exotic annual grasses are preferred for insect vectors of virus (Ingwell and Bosque-Perez, 2015). Moreover, perennial ryegrass (*Lolium perenne* L.) has been shown to be a source of BYDV in the field (Catherall et al. 1966). Corn, which is also an expanding crop in Idaho (Marshall and Rashed 2014), may harbor considerable number of cereal aphids, which could potentially colonize emerging wheat in early autumn; alternatively corn residue in the fields may also increase incidence of BYDV in winter wheat (Cowger et al. 2010). The occurrence of BYDV in corn and some species of annual weed grasses, may suggest their role as sources of BYDV. Knowledge about potential BYDV reservoirs, species of aphid vectors, their timing of appearance in corn and grassy weeds and the time of their movement onto cultivated cereals are critical in developing effective BYDV management practices at the regional level.

REFERENCES:

Catherall, P. L. 1966. Effects of barley yellow dwarf virus on the growth and yield of single plants and simulated swards of perennial rye-grass. *Annals of Applied Biology* 57:155–162.

Cowger, C., Weisz, R., Anderson, J. M., and Horton, J. R. 2010. Maize debris increases barley yellow dwarf virus severity in North Carolina winter wheat. *Agronomy Journal* 102:688-696.

D'Arcy, C. J. 1995. Symptomology and host range of barley yellow dwarf. In: D'Arcy CJ, Burnett PA, eds. Barley yellow dwarf: 40 Years of Progress. St Paul, MN, USA: The *American Phytopathological Society* 9–28.

Hadi, B. A. R. 2009. Aphid vectors and grass hosts of barley yellow dwarf virus and cereal yellow dwarf virus in Alabama and Western Florida. https://etd.auburn.edu/handle/10415/2018

Ingwell, L. L., and Bosque- Pérez, N. A. 2015. The invasive weed *Ventenata dubia* is a host of barley yellow dwarf virus with implications for an endangered grassland habitat. *Weed Research* 55: 62 –70.

Marshall, J.M., and Rashed, A. 2014. Barley yellow dwarf virus in Idaho cereal crops. University of Idaho Current Information Series 1210, Moscow, Idaho. https://www.cals.uidaho.edu/edcomm/pdf/CIS/CIS1210.pdf

IDAHO WHEAT COMMISSION - BUDGET FORM

Allocated by	Idaho Wheat Commission	during FY 2016	$	9,980
Allocated by	Idaho Wheat Commission	during FY 2017	$	11,668

REQUESTED FY2018 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	$ -	$ 4,000	$ 1,636	$ 2,400	$ 3,900	$ -	$ 11,936

TOTAL BUDGET REQUEST FOR FY 2018: $ 11,936

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	Rashed	*Hutchinson*	*Bosque-Perez*	*Marshall*
Salary	$ -	$ -	$ -	$ -
Temporary Help	$ 2,500	$ 1,500	$ -	$ -
Fringe Benefits	$ 1,023	$ 614	$ -	$ -
Travel	$ 1,000	$ 500	$ -	$ 900
Operating Expenses	$ 3,300		$ 600	$ -
Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ 7,823	$ 2,614	$ 600	$ 900

Total Sub-budgets $ 11,936

Explanatory Comments: (see FY2018 Guidelines for definition)

11.21.2016 - Version

ANNUAL REPORT

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ACCOMPLISHMENTS

The second year (2016-2017) of our survey, aimed at identifying potential reservoirs of the barley yellow dwarf virus (BYDV) in grassy weeds, and evaluating the potential role of corn in BYDV spread, was carried out between July and November. In our two-year survey, aphid monitoring was conducted in 41 fields, in coordination with corn and cereal growers, in central and eastern Idaho. Monitored fields included corn adjacent to winter wheat, corn adjacent to potato and sugar beet, and winter wheat away from corn production. BYDV presence had previously been reported in central and eastern Idaho between 2013 and 2016. Sticky traps were replaced every 3 to 4 weeks. In 2016, yellow pan traps containing glycol were also placed in all fields to facilitated identification of the present aphid species. Over the past two years, samples of wheat, barley, corn, and different species of weeds such as common lambsquarters (*Chenopodium album*), downy brome (*Bromus tectorum*), foxtail barley (*Hordeum jubatum*), green foxtail (*Setaria viridis*), orchardgrass (*Dactylis glomerata*), quackgrass (*Elymus repens*), redroot pigweed (*Amaranthus retroflexus*), reed canary grass (*Phalaris arundinacea*), ryegrass (*Lolium perenne*), western salsify (*Tragopogon dubius*), wild oat (*Avena fatua*), kochia (*Bassia scoparia*), smooth bromegrass (*Bromus inermis*), feral rye (*Secale cereale*), wheatgrass sp. present in field corners (CRP), and growing in roadside ditches and along corn and winter wheat field edges were collected and tested for BYDV. For the first time we identified foxtail barley as a BYDV reservoir in Idaho, and we are preparing a research paper quantifying the potential role of this weed species in the virus spread. Results have been presented in cereal schools and departmental seminars.

Aphid population dynamics and identification

Aphid populations were monitored between July and November 2015 and 2016. We completed aphid counts on the sticky traps collected during the 2015 growing season. Aphids from 2016 sticky traps are being counted, and data will be presented at the cereal schools and research review sessions. In 2015, higher densities of aphids were observed in October within corn fields in all locations. In control fields including sugar beet fields adjacent to the corn, and winter wheat fields located far from corn production, aphid densities remained relatively low. In potatoes however high density of aphids were recorded. We are currently in the process of identifying the aphid species collected in our yellow pan traps. Our 2015 data indicated that following corn maturation in October and November aphid numbers increase in cereal fields, suggesting potential movement from corn into nearby winter wheat and winter barley fields. During 2016, aphids from corn fields in all trapping locations were transported to greenhouse to evaluate BYDV transmission success to wheat, by the bird-cherry oat aphids naturally colonizing corn.

Survey of BYDV in corn and wild host in field

All corn samples (leaf tissue; N=11) from a single field in Kimberly tested positive for BYDV in 2015 and 2016. However, 43% (N=14) of the corn samples collected from Paul area tested positive, 20% (N=5) of the corn samples from Buhl, and 17 % (N=6) of the corn samples from American Falls were BYDV positive in 2015. In 2016, BYDV was detected in 50% (N=12) of collected corn samples in Buhl, in 12.5% (N=8) of the corn samples from Paul and in 8.5% (N=12) of the collected corn leaves from American Falls area. In 2015, 13.5% of the winter barley samples (N=15) planted next to corn in single field in Buhl, tested positive for BYDV. In 2016, only wheat sample (N=8) from one field in Buhl showed 50 % BYDV infection and the remaining wheat fields were tested BYDV negative. In each location, leaf tissue for BYDV test were collected from corn, wheat and barley plants surrounding the traps.

A total of 15 plant species collected (listed above) and only the foxtail barley (*Hordeum jubatum*) samples from Magic Valley (ID) tested positive for BYDV infection. Additional samples collected in eastern Idaho are currently being tested for BYDV. RT-PCR product were sequenced, and the obtained result showed 93 to100 % similarity to BYDV-PAV. This finding confirmed that foxtail barley was infected with PAV species of BYDV. This is also the first report of BYDV-PAV infection in foxtail barley in Idaho. The presence of BYDV-PAV in foxtail barley indicates that this weed may potentially serve as a reservoir for the pathogen, and subsequently, facilitate BYDV spread into cereals.

To evaluate the potential role of grassy weeds and corn to act as a source of BYDV-PAV in southern Idaho, transmission essays were also performed. Downy brome, foxtail barley, green foxtail and corn were planted in the greenhouse and exposed to the veriliferous bird cherry-oat aphids from our BYDV-infected greenhouse colony. Four weeks after inoculation, leaf tissues were removed from all plants and tested by qPCR to confirm BYDV infection. Then, the bird cherry- oat aphids were placed on the infected plants for a 5-day acquisition access period. Aphids were then moved to two-week old healthy wheat seedlings (WB-Junction) to evaluate the likelihood of successful transmission.

A significant variation was observed in aphid ability to inoculate host plants (binary logistic regression, Wald = 17. 03, $P = 0.001$). BYDV infection was detected in 75% (N=20) of inoculated foxtail barley, 87% (N=23) of downy brome, 50% (N=26) of green foxtail and 54 % (N=61) of corn (Fig.1A, B). BYDV titer was not statistically different among the evaluated host plants after 4 weeks of incubation (ANOVA: P = 0.932). There was a significant effect of time replicate ($P < 0.001$).

When grassy weeds and corn were used as the virus source, transmission success from foxtail barley, green foxtail, downy brome, and corn to winter wheat was 86%, 100%, 91% and 58%, respectively (Fig. 2). Although variations were observed, statistically transmission success to wheat seedlings was not affected by the source plant species (Wald = 0.316, Df = 3, $P = 0.957$). Additional replicated may help to detect potential variations in the future essays that are scheduled for 2017-2018 season. This indicated that all these host plants may serve as a source of the virus in the area.

In addition to conducting transmission experiments and surveys of aphid movement and BYDV reservoirs, we tested nearly 150 samples for our winter cereal producers during 2015 and 2016 outbreaks, free of charge.

PROJECTIONS

An area wide sampling of wild grassy hosts is planned for March and August 2017. These include sampling approximately 100 field sites from southern Idaho, as well as selected sites in northern Idaho. This expansion is needed to develop BYDV hotspot maps for southern Idaho. The list of potential virus hosts is also expected to expand. Our transmission data will help to estimate the likelihood of the identified wild hosts to serve as a BYDV source for cultivated grasses. We will also continue to monitor aphid movement into cereals from corn crop to see weather recent BYDV outbreaks can be explained be the expansion in corn acreage. Our experiments would also help to understand if there are differences in BYDV transmission success by the bird cherry-oat aphids naturally colonizing corn and wheat. Furthermore, results will be used in making management decisions, including weather late-season insecticide application in corn would help to reduce aphid and BYDV spread into emerging winter cereals.

PUBLICATIONS

A manuscript comparing BYDV transmission success from grassy weeds to wheat is currently in preparation. A CIS publication will be prepared at the end of our study. The article will provide information source of BYDV infection, their importance in BYDV spread/outbreaks, and aphid's movement pattern from corn to the nearby winter wheat.

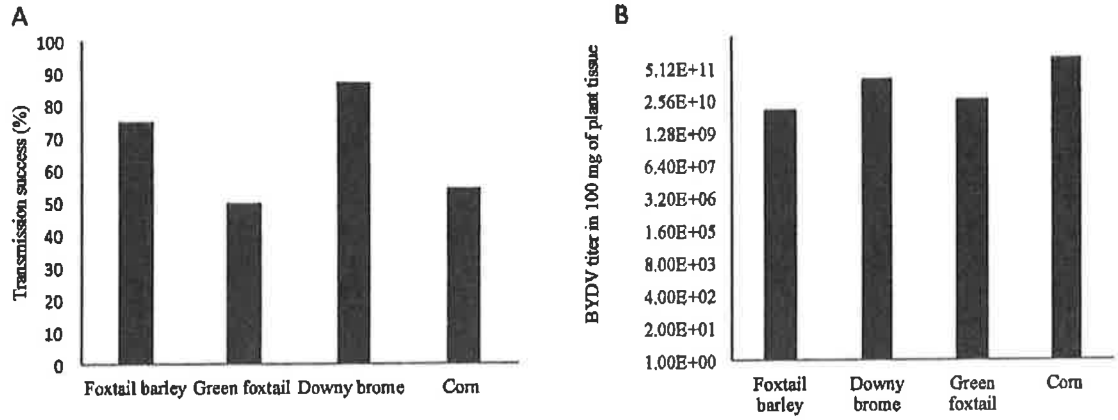

Figure 1: Percentage of the infected plants after exposure to 10 BYDV positive bird cherry-oat aphids (A). BYDV titer in infected plants after 4-weeks of incubation (B).

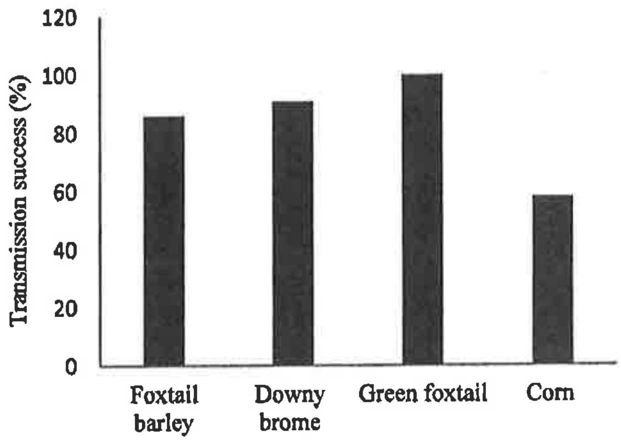

Figure 2: Percent transmission success from evaluated grassy weeds and corn to WB-Junction wheat