PROJECT NO:	BJKU49, BJKU50, BJKU51, BJKU52
TITLE:	The Importance of Corn and Grassy Weeds in BYDV Spread
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JUSTIFICATION: Barley yellow dwarf virus (BYDV) is an important viral pathogen of cereal crops. The virus has a broad host range that includes many perennial weeds, forage grasses, and other crops such as corn (*Zea mays*) and rice (*Oryza sativa* L.) (De Wolf 2010). Several species of cereal aphids are responsible for spreading BYDV, and among them the Bird Cherry-Oat Aphid (*Rhopalosiphum padi*) is known to be one of the most efficient species for vectoring the virus. Cereal aphids contributed to the 2012-2013 (Marshall and Rashed, 2014), and most recently, 2014-2015 outbreak of BYDV in south-central Idaho.

A BYDV survey of grasses in northern Idaho pastures showed that a large percentage of common plant species can host BYDV (Ingwell and Bosque-Pérez, 2015). Moreover, anecdotal observation during 2012-2013 and 2014-2015 outbreaks indicated that some weedy grasses near BYDV–infected winter barley in Burley Idaho, showed reddening/yellowing symptoms typical of BYDV infection. These observations raise the question as to whether or not weedy grasses can act as source for BYDV and its aphid vectors. To address this question, during the 2015 crop season (first year of this study), yellow sticky traps were placed in 20 corn and neighboring winter wheat fields, as well as in winter wheat away from corn (control fields). Traps have been replaced on regular basis and are being screened for aphid presence. In year 2 (2016-2017 season), we will continue to identify additional monitoring sites to monitor aphid movement from corn, and grassy weeds, and native grasses into cultivated cereal fields. To date, we have shown that 100% of the weed foxtail barley (*Hordeum jubatum*) collected from Magic valley tested positive for BYDV. However, none of the downy brome (*Bromus tectorum*) samples collected during the first year of our study tested positive for BYDV. Likewise, all sampled wheatgrass sp. planted in field corners, and growing in roadside ditches and along field edges (native grasses) tested negative for BYDV. A vast majority of these samples were tested with ELISA. Our recent analyses revealed that corn samples, which tested negative for BYDV using ELISA, showed detectable infection levels with the more sensitive qPCR. In relation to this, under greenhouse conditions and using the PCR approach, 25% of green foxtail (*Setaria viridis*), and 43% of corn infested with the infective bird cherry-oat aphid tested positive for BYDV using RT-PCR and qPCR, respectively. Therefore, in 2016-2017, qPCR will be used for a subset of collected samples from each location to validate ELISA findings in 2016-2017.

To date, results of our greenhouse studies, and field survey of BYDV host have indicated that corn, green foxtail, and foxtail barley could act as a reservoir for BYDV in south-central Idaho. Transmission studies based on our findings are currently in progress.

Our most recent analyses of samples collected fall 2015 from winter cereals in south-central Idaho revealed the presence of BYDV, indicating the possibility for another year of this virus in the region. As BYDV continues to impact our agricultural systems, our monitoring of aphid movement and screening of alternate hosts as reservoirs for both virus and aphids will provide valuable information that will lead to developing region-specific recommendations for sustainable and integrated management of the disease.

Objectives:

I: Conduct a comprehensive field survey to determine BYDV occurrence in corn and grassy weeds surrounding wheat production areas throughout the year at different locations in south-central Idaho

II. Determine pattern of disease incidence and progress within wheat fields neighboring potential reservoirs (corn and naturally occurring grassy hosts)

III. Study the efficiency of transmission from corn and grassy weeds, especially green foxtail, and foxtail barley which commonly were found to host BYDV, to wheat under controlled greenhouse conditions

PROCEDURES:

Objective I: We will continue our plant sampling within, and near, 20 wheat fields neighboring corn and/or open pastures in central and eastern Idaho where BYDV has been reported in the past. Collected samples will be tested by qPCR. Additional fields are expected to be added to our monitoring program during 2016-2017.

Objective II: We will continue to monitor aphid movement into wheat using yellow sticky traps. Traps will be placed on the edge of selected corn fields and within wheat fields in different distances from the edge facing corn. Traps will be replaced overtime to monitor insect movement from corn into wheat. Number of aphids is expected to rise, if insects started to move into emerging winter wheat fields, as the corn crop matures or being harvested. Leaf samples will be collected from wheat plants surrounding the traps (at different distances from the field edge) to measure the rate of BYDV occurrence in relation to distance from potential reservoirs.

Objective III: To study transmission efficiency, corn, green foxtail, and foxtail barley seeds will be planted in the greenhouse. Ten viruliferous bird cherry- oat aphids from a BYDV positive laboratory colony will be used to inoculate weeds and corn plants. Viruliferous aphids will be allowed to feed for a 72-hrs period (inoculation access period or IAP). When the IAP period has elapsed, aphids will be removed with insecticide application. Infected plants will be maintained in the greenhouse for a minimum of 4 weeks (26-30 C, 70% RH). Using leaf-clip cages, aphids from a BYDV-free colony will be placed in groups of 3-5 on BYDV infected corn and weeds (see above) for minimum of 48-hr (acquisition access period or AAP). Then, insects will be removed to healthy wheat plants at 3-4 leaf stage for a 72-hr IAP. All of the source plants (BYDV-infected weeds) will be tested for BYDV presence and titer (qPCR). Aphids will then be removed and tested for infection status (virus incidence and titer) individually. After a 4-week period all experimental wheat plants (target plants) will be sampled for BYDV presence. This approach would allow us to evaluate transmission success from potential reservoirs to emerging wheat, first, by quantifying acquisition success from green foxtail, foxtail barley, and corn, and then by determining successful rates of inoculation into wheat.

DURATION: This study is expected to complete in 3 years. This would be the 2nd year of this 3-year proposal.

COOPERATION: This is a collaborative project between Dr. Rashed, Dr. Hutchinson, Dr. Marshall and Dr. Bosque-Pérez's laboratories in cooperation with several regional cereal growers.

ANTICIPATED BENEFITS/EXPECTED OUTCOMES/INFORMATION TRANSFER:

This research will result in determining the potential role of corn and naturally occurring grassy weeds, in BYDV epidemiology in south- central Idaho. As result of this work, to date, corn, green foxtail, and foxtail barley have been identified to host BYDV. This proposal was expanded to examine more key host-plant species for BYDV and aphids, and determining aphid movement within wheat fields adjacent to corn fields. Our first year collaborative efforts resulted in confirming foxtail barley as a BYDV host in south-central Idaho. Testing additional grassy weed species would help growers in their BYDV management decisions (e.g. seed treatment and/or planting date) to limit damage in high risk areas/years. If corn proved to be a significant source of BYDV in field, then late-season insecticide applications (in corn) may be suggested to reduce aphid numbers and movement to emerging winter wheat. Results will be communicated to growers and scientific community through cereal schools, CIS publication, conferences and refereed journal publications.

LITERATURE REVIEW:

BYD is one of the most destructive diseases of cereal crops worldwide and its host range includes a wide range of cultivated and non-cultivated grasses (Power et al. 1994). While barley and wheat yields may be severely affected by BYDV infection, alternate hosts such as corn (Halbert et al.1995) and/or other grasses (Hadi 2009) may play a role as a reservoir of the pathogen in the absence of wheat and barley (Stoner 1977). Knowledge of BYDV ecology and potential reservoirs for the virus and its aphid vectors would help to establish sustainable management practices that are based on removing green bridges, reducing aphid populations, and adjusting planting times.

It has been reported previously that corn residue in the field can increase the incidence of BYDV in North Carolina's winter wheat (Cowger et al. 2010). The occurrence of BYDV in corn and some species of annual weed grasses has also been documented in Europe suggesting their roles as major sources of BYDV affecting winter cereals. Moreover, it has been shown that corn may harbor more aphids, which can colonize wheat fields in early autumn. Such findings also show how manipulations of landscape structure may impact existing agro-ecological equilibriums contributing to pest outbreaks (Vialatte et al. 2006).

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Halbert, S.E. Forster, R.L. Sandvol, L.E. Quisenberry, S.S. Hein, G.L. 1995. Fall pest problem of winter wheat. University of Idaho, Cooperative Extension System. EXT 780. pp:1-8.

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Power, A.G, and Remold, S.K. 1994. Incidence of Barley yellow dwarf virus in wild grass populations: implication for biotechnology risk assessment. http://www.isb.vt.edu/brarg/brasym96/power96.htm
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Vialatte, A. Simon, J.C. Dedryver, C, A. Fabre, F. Plantegenest, M. 2006. Tracing Individual Movements of Aphids Reveals Preferential Routes of Population Transfers in Agroecosystems. *Ecological Society of American* 16:839-844.

IDAHO WHEAT COMMISSION - BUDGET FORM

Allocated by	Idaho Wheat Commission	during FY 2015	$	-
Allocated by	Idaho Wheat Commission	during FY 2016	$	10,180

REQUESTED FY 2016 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	$ -	$ 4,000	$ 1,568	$ 2,500	$ 3,600	$ -	$ 11,668

OTHER RESOURCES (not considered cost sharing or match Salary/operation
6553

TOTAL OTHER RESOURCES $ 6,553

TOTAL PROJECT ESTIMATE FOR FY 2017:	$ 11,668	$ 6,553	$ 18,221
	(Requested)	*(Other)*	*(Total)*

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	Rashed	*Hutchinson*	*Bosque-Perez*	*Marshall*
Salary	$ -	$ -	$ -	$ -
Temporary Help	$ 2,500	$ 1,500	$ -	$ -
Fringe Benefits	$ 980	$ 588	$ -	$ -
Travel	$ 1,000	$ 600	$ -	$ 900
Operating Expenses	$ 3,200		$ 400	$ -
Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ 7,680	$ 2,688	$ 400	$ 900

Total Sub-budgets $ 11,668

10.7.2015 - Version

ANNUAL REPORT

PROJECT NO: BJKU49, BJKU50, BJKU51, BJKU52

TITLE: The importance of corn and grassy weeds in BYDV spread

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ACCOMPLISHMENTS:

This project was started in August 2015. The first step was finding growers that had planted (or were going to plant) winter wheat fields adjacent to corn in different counties in central and eastern Idaho, especially in locations where BYDV has been reported in the past. More than 100 traps have been placed on the edge of selected fields and within winter wheat fields neighboring corn and/or open pastures in different locations across Idaho. These locations included Minidoka, Twin Falls, and Power counties. For every location, winter wheat fields not neighboring any corn were monitored and sampled as controls. The traps were replaced every 2-3 weeks, and leaf samples were collected from wheat plants surrounding the traps (at different distances from the field edge) to measure the rate of BYDV occurrence in relation to distance from potential virus reservoirs. All collected leaf samples are being tested for BYDV.

Besides corn, wheat and barley, different species of grassy weeds such as common lambsquarters (*Chenopodium album*), downy brome (*Bromus tectorum* L.), foxtail barley (*Hordeum jubatum*), green foxtail (*Setaria viridis*), orchardgrass (*Dactylis glomerata* L.), quackgrass (*Elytrigia repens*), redroot pigweed (*Amaranthus retroflexus*), reed canary grass (*Phalaris arundinacea*), ryegrass (*Lolium perenne*), western salsify (*Tragopogon dubius*), wild oat (*Avena fatua*), and wheatgrass sp. planted in field corners, growing in roadside ditches and along field edges were collected from areas surrounding cereal fields to be tested for BYDV.

Different species of cereal aphids are responsible for spreading BYDV. Bird Cherry-Oat Aphid is one of the most efficient species for vectoring the virus, and contributed to the 2012-2013 outbreak of BYDV in south-central Idaho. To evaluate the significance of wild hosts and corn in BYDV epidemiology in Idaho, we also established a greenhouse study. Viruliferous Bird Cherry-Oat aphids were used to inoculate downy brome (*Bromus tectorum*), foxtail (*Setaria* spp.), western wheatgrass (*Pascopyrum smithii* (Rydb.), Siberian wheatgrass *Agropyron fragile* (Roth), and corn (*Zea mays* L.) in the greenhouse. These plants will be used as a source for BYDV to quantify vector efficiency in acquiring the virus, and later their success rate in inoculating the winter wheat (WB-Junction) seedlings. This experiment is currently ongoing and the results are expected to be available for the cereal schools.

Aphid population dynamics

Through this survey, yellow sticky traps from 20 fields of corn and neighboring winter wheat, as well as traps from winter wheat away from corn (control field) have been collected between August and November. The number of cereal aphids on traps will be counted during December. Aphid counts on traps can provide a relative measure of aphid density, fluctuations in the number of adults caught throughout sampling dates, and whether proximity to corn fields can impact aphid numbers and movement within winter cereals.

Wild host identification

Plant samples were collected from the edges of the fields in central and eastern Idaho. The collected plants included corn, wheat, and barley, and several grassy weed species (listed above) collected from central and eastern Idaho. To date, only samples collected from south-central Idaho have been tested for BYDV by RT-PCR. The remaining samples are set to be tested in upcoming weeks for BYDV. South-central Idaho is frequently impacted by BYDV and our evaluations of plants collected from that area are expected to provide a clear image of potential wild hosts of BYDV. Interestingly, 100% of the foxtail barley (*Hordeum jubatum*) collected from Magic valley tested positive for BYDV. Downy brome also is known to host BYDV however, to date, none of the downy brome samples collected from our study sites tested positive for the virus. Likewise, wheatgrass sp. planted in field corners, and growing in roadside ditches and along field edges (native grasses) tested negative for BYDV. It is also known that BYDV ceases multiplication at higher temperature (>80F). Therefore, collecting samples later in the season may have contributed to our failure to successfully detect BYDV. Our 2016 samplings will start in the spring and would provide a more accurate assessment of potential wild hosts for BYDV. We have already tested several winter wheat samples planted this fall for local growers that tested positive, indicating another year of BYDV presence in this upcoming crop.

Greenhouse transmission experiments

Wild grasses including downy brome (*Bromus tectorum*), foxtail (*Setaria* spp.), western wheatgrass (*Pascopyrum smithii* (Rydb.), and Siberian wheatgrass *Agropyron fragile* (Roth), and corn (*Zea mays*) were exposed to veriliferous bird cherry-oat aphids from our infective colonies. After 4 weeks of incubation, all plants were tested by ELISA to examine the potential of these plant species to host BYDV-PAV. Only 4 out of 16 foxtail (*Setaria* spp.) tested positive for BYDV using RT-PCR. Our failure to detect the pathogen in other species might have been due to low virus titer. Since qPCR sensitivity is expected to be greater than ELISA in detecting the pathogen, inoculated plants which were negative in ELISA will be retested using qPCR. This experiment will be repeated in January 2016.

So far, results of the greenhouse BYDV transmission study and field survey of BYDV hosts have indicated that foxtail (*Setaria* spp.) and foxtail barley (*Hordeum jubatum*) can act as a reservoir for BYDV in the region. Additional BYDV hosts will be identified with testing of samples stored for laboratory analysis and samples which will be collected in the spring of 2016.

Next Steps

To study aphid movement and the role of corn in BYDV spread, more fields will be surveyed in the upcoming season. We are working closely with growers and county extension educators to identify new corn/wheat planting sites across the region to continue our monitoring program.

Potential wild hosts and corn plants currently inoculated in our greenhouse will be tested for BYDV using qPCR. This will enable us to detect the virus even if present in low titer. Virus quantification will also allow us to assess the relationship between pathogen titer and the rate of BYDV transmission. Since foxtail barley has been confirmed as one of the regional wild hosts of BYDV, our winter greenhouse study will focus on evaluating its impact in the disease epidemiology. In addition, transmission efficiency of the virus by the bird cherry-oat aphid will be evaluated and results will be available by the end of January for presentation at the cereal schools. This study will also include downy brome as this species is known to be a BYDV host in other regions in Idaho. In addition, more corn and weedy grasses will be tested with RT-PCR to quantify BYDV incidence. Plants have been inoculated and transmission experiments are set to be conducted in January 2016.

In summary, we intend to:

- Continue sampling fields near corn for aphid movement and role of corn in the disease epidemiology
- Test wild plant species and corn inoculated with viruliferous aphids in the greenhouse
- Determine relationship between virus titer in foxtail barley and subsequent transmission to other crops

PROJECTIONS

Identifying the role of corn and other grasses as BYDV hosts will help improve the efficiency of our monitoring program and management practices. Furthermore, results of this research could determine if insecticide application in corn and field borders towards end of the season will reduce aphid number and movement to emerging winter wheat.

PUBLICATIONS

A CIS publication will be prepared at the completion of this study (within 2 years). This article will provide information on the potential role of corn and naturally occurring grassy weeds in BYDV epidemiology, and identify of new hosts of BYDV in central Idaho. Information on BYDV/aphid presence and prevalence in wild vegetation surrounding wheat production would also help growers to take preventive measures (e.g. seed treatment and/or planting date) to limit damage in high risk areas and years.

Fig .1. Yellow sticky trap in corn field

Fig.2. Three-row yellow sticky traps in a wheat field neighboring a corn field