Survey of *Barley Yellow Dwarf Virus* and *Cereal Yellow Dwarf Virus* on Three Perennial Pasture Grasses in Florida[1]

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Abstract *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* (CYDV) are two important aphid-vectored viruses of small grains worldwide. In the Southeastern U.S., there is an absence of wheat, oat and barley during summer months. Thus, the availability of alternative summer hosts for the viruses and their vectors in the field is potentially critical for yellow dwarf epidemiology in the southeast. A 2-year survey of bahiagrass (*Paspalum notatum* Flugge), limpograss [*Hemarthria altissima* (Poir) Stapf & Hubb.], and eastern gamagrass (*Tripsacum dactyloides* L.) populations from the North Florida Research and Education Center, Marianna, FL. showed that bahiagrass and limpograss can act as alternative summer hosts of BYDV-PAV and CYDV-RPV. Additionally, bahiagrass also was shown to harbor BYDV-MAV. The finding of BYDV and CYDV on bahiagrass and limpograss indicates the potential of these perennial pasture grasses to act as "green bridges" between small grain seasons for yellow dwarf viruses. For bahiagrass and limpograss to act as sources of inoculum in yellow dwarf epidemiology, one or more aphid species will have to transmit the virus from these grasses to commercial small grains. However, very few aphids were collected and no known B/CYDV vector was found on these grasses in the 2 years of sampling. *Sipha flava* (Forbes), an aphid not listed as B/CYDV vector, was the only species collected on bahiagrass. Additionally, in B/CYDV transmission studies reported here, winged *R. padi* did not survive on bahiagrass and failed to transmit B/CYDV from bahiagrass to oats or from oats to bahiagrass.

Key Words BYDV, CYDV, bahiagrass, limpograss, *Sipha flava*

Yellow dwarf (YD) has been reported worldwide and is considered a serious disease of wheat (Plumb 1983). The causal pathogens of yellow dwarf are viruses from the family *Luteoviridae*, including *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* (CYDV). Currently, there are 3 recognized species of *Barley yellow dwarf viruses* (*Barley yellow dwarf virus*-PAV, *Barley yellow dwarf virus*-MAV and *Barley yellow dwarf virus*-PAS) assigned to genus *Luteovirus*. One species of *Cereal yellow dwarf virus*, *Cereal yellow dwarf virus*-RPV, is assigned to genus *Polerovirus*. Three other species in the virus complex (*Barley yellow dwarf virus*-GPV, -RMV and –SGV) are yet to be assigned into a genus (ICTV 2009).

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Barley yellow dwarf virus and *Cereal yellow dwarf virus* are known to exclusively infect plants from Family Poaceae. A comprehensive review of the B/CYDV host range was given by D'Arcy (1995), in which it was stated that the yellow dwarf viruses can infect "... more than 150 species in 5 of 6 subfamilies of the Poaceae and in 11 of the 25 tribes." In regions where economically important hosts such as wheat, barley or rice are only planted in a specific portion of a year, various lawn, weed, pasture and range grasses may act as alternative hosts in which the viruses and vectors survive, facilitating the introduction of the viruses to new plantings of commercial hosts (D'Arcy 1995).

Since the publication of the B/CYDV host list by D'Arcy (1995), few new grass species have been reported as hosts (Table 1). Some of these plants are introduced species in the U.S., whereas some are native grasses (USDA-NRCS 2011). The finding of B/CYDV on these grasses illustrates the potential of plants in grasslands as alternative hosts for the viruses causing YD. Three of the host plants listed in Table 1 – common reed (*Phragmites communis* Trin.), weeping lovegrass [*Eragrostis curvula* (Schrad.) Nees] and blue wildrye (*Elymus glaucus* Buckley) – were listed as nonhosts of B/CYDV in earlier reports (Bruehl and Toko 1957, Griesbach et al. 1990, Guy et al. 1987, Oswald and Houston 1953). Discrepancies among these reports may have stemmed from various factors including differences in virus strains, inoculum pressure, and the possible biotypes of vector species (D'Arcy 1995).

Malmstorm and Shu (2004) observed bahiagrass with YD-like symptoms in Florida but were unable to detect B/CYDV using serological tests. BYDV-PAV was detected in bahiagrass grown in Florida using multiplex PCR (A. Blount and J. Anderson, unpub. data). The goals of the study reported herein was to confirm the presence of B/CYDV in bahiagrass (*Paspalum notatum* Flugge) and 2 other summer pasture grasses – limpograss [*Hemarthria altissima* (Poir) Stapf & Hubb.] and eastern gamagrass (*Tripsacum dactyloides* L.) – and to investigate their potentials as an inoculum source of B/CYDV in winter wheat infection.

Materials and Methods

Field collection. Variety trial plots of pasture grasses at the North Florida Research and Education Center in Marianna, FL (30.46°N, -85.13°W), were surveyed in 2007 and 2008. The plots were surveyed for aphids once a month between June and August. Plant samples were collected once each year between June and August. Three species of pasture grasses were available for sampling – bahiagrass, eastern gamagrass, and limpograss. Eight cultivars of bahiagrass were sampled: "Pensacola", "Tifton 9", "TifQuick", "UF-Riata", "AU Sand Mountain", "Paraguay 22", "Argentine", and Tifton 7. The first 5 of these cultivars are of diploid type, whereas the last 3 are of tetraploid type.

Ten leaves of each grass species that showed typical symptoms of B/CYDV infection, namely yellowing or reddening, were collected per plot. Plots without symptomatic leaves were not sampled. Collected leaves were tested for virus presence and strain identification using triple antibody sandwich enzyme-linked immunosorbent assay (ELISA) (Agdia Inc., Elkhart, IN) according to the manufacturer's direction. Plant sap was extracted from each leaf sample with the addition of 1 ml of general extraction buffer (prepared as recommended by the manufacturer, Agdia, Inc.) using a motorized leaf squeezing apparatus. A 100-µL aliquot of extract from each sample was

Table 1. Hosts of barley yellow dwarf viruses reported since D'Arcy (1995).

Tribe and species	Common name	Reference
Arundineae		
Phragmites communis Trin. *	Common reed	Ilbagi 2006
Avenae		
Koeleria macrantha (Ledeb) J.A. Schultes	Prairie Junegrass	Malmstorm et al. 2005
Cynodonteae		
Chloris truncata R. Br.	Australian fingergrass	Hawkes and Jones 2005
Chloris virgata Sw.	Feather fingergrass	Hawkes and Jones 2005
Eragrostideae		
Eragrostis curvula (Schrad.) Nees**	Weeping lovegrass	Hawkes and Jones 2005
Oryzeae		
Erharta calycina Sm.	Perennial veldt grass	Hawkes and Jones 2005
Paniceae		
Pennisetum clandestinum Hochst. ex Chiov.	Kikuyu grass	Hawkes and Jones 2005
Setaria viridis (L.) P. Beauv. †	Green bristlegrass	Remold 2002
Paniceae		
Setaria pumila (Poir.) Roem & Schult. ssp. pumila	Yellow foxtail	Remold 2002
Urochloa panicoides P. Beauv.	Panic liverseed grass	Hawkes and Jones 2005
Stipeae		
Nassella pulchra (Hitchc.) Barkworth	Purple needlegrass	Malmstorm et al. 2005
Triticeae		
Elymus glaucus Buckley †	Blue wildrye	Malmstorm et al. 2005
Elymus elymoides (Raf.) Swezey	Squirreltail	Malmstorm et al. 2005
Elymus multisetus M.E. Jones	Big squirreltail	Malmstorm et al. 2005

* Reported in Guy et al. (1987) as a nonhost of BYDV.
** Reported in Griesbach et al. (1990) as a nonhost of BYDV.
† Reported in Oswald and Houston (1953) and Bruehl and Toko (1957) as nonhosts of BYDV.

added to a microtiter plate well. Substrate reactions (p-nitrophenyl phosphate at 1 mg/ml in 10% diethanolamine, pH 9.8) were allowed to develop for 30 min before absorbance values were recorded using a BioTek ELx 800 Microtiter plate reader (Biotek. Inc., Winooski, VT) at 405 nm. In 2007, the samples were tested for the presence of BYDV-PAV and CYDV-RPV. In 2008, the samples were tested for BYDV-MAV, BYDV-PAV and CYDV-RPV. Known wheat-based negative and positive controls obtained from Agdia Inc. were added to each microtiter plate. A sample was considered positive for the presence of virus if the ELISA absorbance value was greater than 3X the average of the healthy controls on a given plate (Sutula et al. 1986).

Aphids were sampled alive from the plants and assayed for B/CYDV infectivity. The infectivity assay was conducted in accordance with the methods described by Chapin et al. (2001) and Halbert et al. (1992). In the first 2 sampling attempts, the aphid survey was conducted by visually searching the plots for aphids. However, no aphids were found using this method. Subsequently, live aphids were successfully collected by sweeping the plots using a sweep net. Each collected aphid was placed directly in a glass tube containing a 7 - 14-day-old "California Red" oat seedling on moist cotton or tissue paper. The tubes containing aphids and oat seedlings were incubated under room temperature and ambient light to allow for an inoculation access period. After 48 - 72 h of an inoculation access period, all aphids were removed from the tubes, preserved in 80% ethanol and identified to species according to the key found in Blackman and Eastop (2000). The oat seedlings were treated with lambda-cyhalothrin insecticide to kill all residual nymphs, then potted and incubated in a growth room with the temperature set at 25°C and a 12/12 h light/dark cycle. By the end of 2 - 3 wks of incubation period, the 3 oldest leaves were harvested from each plant and the leaf tissue composites were subjected to ELISA using commercial antibodies produced by Agdia Inc. (Elkhart, IN) as described above.

Aphid colony establishment. An aphid colony was started from a single *R. padi* collected at Sand Mountain Research and Extension Center, Crossville, AL that was found to be viruliferous with BYDV-PAV. The aphid was reared on oat seedlings of 'California Red' cultivar in a growth chamber with temperature set at 15°C and a 12/12 h light/dark cycle. The relatively low temperature was maintained to induce winged aphid production within the colony. At any given time, 6 potted oat plants were kept as a food source and virus reservoir for the colony. The plants were changed every 4 - 5 wks by putting 6 new potted oat seedlings in the growth cabinet, interspersed between the older host plants. Aphids moved naturally from the older host plants to the younger ones. The status of virus infection of removed plants was checked by ELISA before the plants were discarded.

After 3 cycles of host plant succession with the first colony, an attempt was made to start a colony of nonviruliferous *R. padi*. A mature aphid was selected and transferred to a virus free oat leaf blade and kept in a sealed Petri dish under room temperature. Every 6 h, the Petri dish was checked for nymphs. Upon the first sighting, the nymphs were transferred to new oat seedlings and kept in a separate growth chamber with temperature and light regimen similar to that of the first colony. After 4 wks, the leaves from plants with the new colony were subjected to ELISA to test for the presence of B/CYDV. As no B/CYDV was found, the colony was maintained by providing new oat seedlings every 4 - 5 wks in the same manner as the maintenance of the viruliferous colony. These 2 colonies of *R. padi*, one reared on wheat infected with BYDV-PAV and the other reared on healthy wheat were used in the following experiments.

Experiment 1: Transmission of BYDV-PAV from BYDV-infected bahiagrass to oat by *R. padi* alate. Symptomatic bahiagrass plants were dug from variety plots and transplanted to pots. The presence of BYDV-PAV on these symptomatic potted bahiagrass was ascertained by subjecting the leaves of each plant to ELISA as previously described, and 6 BYDV-PAV infected bahiagrass were selected. These 6 potted BYDV-PAV infected bahiagrass plants were kept in a growth room with temperature set at 25°C and a 12/12 h light/dark cycle. Ten winged *R. padi* reared on healthy oats were transferred to each potted bahiagrass. A pot of healthy oat plants was used as a control, to which 10 winged *R. padi* also were transferred. Each potted plant with aphids was kept in a nylon cage and the aphids were given a 5-d acquisition feeding period. Each aphid was then transferred to a glass tube containing a 7 - 14-day-old 'California Red' oat seedling on moist cotton or tissue paper that served as an indicator plant. After 3 d of inoculation access period, all aphids were removed from the tubes. The oat seedlings were treated with lambda-cyhalothrin insecticide to kill all residual nymphs, then potted and incubated inside nylon cages in the growth room. After 3 wks, the 3 oldest leaves were harvested from each plant and the leaf tissue composites were subjected to ELISA as previously described.

Experiment 2: Transmission of B/CYDV from infected oats to bahiagrass by *Rhopalosiphum padi* alates. Twenty to 30 seeds of the bahiagrass cultivars Argentine and Pensacola were sown per pot, and the pots were kept in a growth chamber with temperature set at 25°C and 12/12 h dark/light cycle. After 6 wks, 4 pots of each bahiagrass variety that showed vigorous growth were selected for inoculation. A pot of healthy 14-day-old oat seedlings was used as a control. Ten winged *R. padi* reared on oat plants infected with BYDV-PAV were transferred to each pot of plants. Each pot with aphids was kept inside a nylon cage in a growth room with temperature set at 25°C and a 12/12 h dark/light cycle. The aphids were given a 3-d inoculation feeding period. After 3 d, lambda-cyhalothrin insecticide was sprayed on the plants to kill the aphids and the plants were kept in the growth room for another 3 - 4 wks. After 3 or 4 wks of incubation period, the leaves from each plant were harvested and subjected to ELISA to detect the presence of BYDV-PAV.

Results

Field-collected leaves of all diploid bahiagrass cultivars and limpograss tested by ELISA were shown to be infected with BYDV-PAV and CYDV-RPV (Table 2). BYDV-MAV was detected from diploid bahiagrass samples in 2008. BYDV-PAV was detected but not CYDV-RPV from tetraploid bahiagrass samples. No BYDV or CYDV was detected from gamagrass samples.

A total of 27 aphids was collected in 2007, but no aphids were collected in 2008. Aphids were found on bahiagrass, but not found on limpograss or gamagrass. All aphids collected in 2007 on bahiagrass were identified as the yellow sugarcane aphid, *Sipha flava* (Forbes). None of the indicator plants showed positive results for BYDV-PAV or CYDV-RPV indicating that none of the aphids collected from bahiagrass were viruliferous with tested YD viruses. However, after 3 d of inoculation feeding period, all of the collected aphids were found dead on the oat seedlings. The sweep net collection method may have accidentally injured the aphids and caused the high mortality rate.

In the first greenhouse experiment, no *R. padi* was recovered alive from bahiagrass after the 5 d feeding period. The aphids on the control (healthy oat plants) were

Table 2. *Barley yellow dwarf* and *Cereal yellow dwarf viruses* detected using ELISA from pasture grass leaf samples collected in North Florida Research and Education Center, Marianna, FL.

	Number of infected samples / total samples				
	2007		2008		
Grass species	BYDV-PAV	CYDV-RPV	BYDV-PAV	CYDV-RPV	BYDV-MAV
Bahiagrass					
Diploid cultivars	20/25	1/25	11/25	4/15	2/15
Tetraploid cultivars	10/15	0/15	2/15	0/15	0/15
Limpograss	10/53	6/53	0/8	0/8	0/8
Eastern gamagrass	0/24	0/24	0/3	0/3	0/3

found alive. The experiment was repeated twice with the acquisition feeding period shortened to 3 d in the second repetition. Even after changing the acquisition feeding period to 3 d, no *R. padi* was found alive in bahiagrass. *Rhopalosiphum padi* appears unable to survive on bahiagrass.

In the second experiment, as in the first one, no *R. padi* was found alive on bahiagrass seedlings after the 5 d feeding period. The aphids on the control oat plants were found alive after the inoculation feeding period. No BYDV-PAV was detected on the bahiagrass leaf samples, but virus was detected in the control oat plants. The experiment was repeated twice with consistent results.

Discussion

Wheat, oat and barley are among the hosts of B/CYDV with high commercial value. Temporal breaks between the seasons of wheat, oat and barley through summer months necessitate the presence of alternative hosts for the YD viruses and vectors to survive in the field (Hewings and Eastman 1995). Aphid vectors introduce yellow dwarf viruses into winter cereals in the fall from these alternative hosts. Irwin and Thresh (1990) pointed out that the inoculum source for B/CYDV fall infection of winter wheat can be local, regional or long distance. Because of the broad host range of B/CYDV, an array of plants, both cultivated and wild, may act as the source for infection to small grain.

Warm-season pasture grasses may act as alternative hosts of B/CYDV. A 3-yr survey of winter wheat fields in the U.K. showed that fields with surrounding grassy areas, including pastures and moorlands, had higher numbers of alate aphids and higher mean levels of B/CYDV incidence (Foster et al. 2004). Of the 13 plants listed in Table 1, weeping lovegrass is used as a forage crop (USDA-NRCS 2011). Bahiagrass and limpograss, the 2 grass species reported here to harbor BYDV, are also used as forage crops. Both bahiagrass and limpograss are adapted to the climate in South Alabama, South Georgia and North Florida. Over 400,000 ha of bahiagrass were

planted in Alabama, and over 200,000 ha in Georgia (Blount 2004). The large areas of bahiagrass and its capability to serve as B/CYDV host may render the grass important as an inoculum source for fall infection to winter cereals by B/CYDV. Both bahiagrass and limpograss were not mentioned in D'Arcy's (1995) list of B/CYDV hosts.

The true importance of a particular warm-season pasture grass as a B/CYDV inoculum source for fall infection of commercial cereals is not clear. In Virginia, after detecting BYDV-PAV on maturing cultivated winter wheat in the spring, Sforza et al. (2001) found BYDV-PAV in tall fescue and BYDV-MAV on orchardgrass adjacent to the studied field the following summer. In the following spring, CYDV-RPV was the predominant yellow dwarf-causing virus detected in cultivated wheat at the same study site, and CYDV-RPV was the sole YD virus found on the adjacent tall fescue and orchardgrass during that summer. These observations showed that virus movement between wheat and pasture grasses may occur although the direction of the movement may not be reciprocal.

The uncertain role of B/CYDV-harboring grasses surrounding cereal fields in yellow dwarf epidemiology is further illustrated by several surveys that found mismatches between the species of yellow dwarf-causing viruses in cereal crops and surrounding wild grasses (Clement et al. 1986, Paliwal 1982, Plumb 1977). An investigation in Indiana demonstrated that grasses surrounding studied fields were infected by CYDV-RPV whereas the wheat fields were predominantly infected by BYDV-PAV (Clement et al. 1986). In England, the virus strains found in cereals often differ in their geographic distribution from those of grasses (Plumb 1977). Paliwal (1982) did not verify the strain of the virus, but reported that the grasses collected from Ontario and Quebec mainly harbor yellow dwarf virus specifically transmitted by *R. padi*, whereas the winter wheat was predominantly infected by yellow dwarf virus specifically transmitted by *S. avenae*. Masterman et al. (1994) reported that aphids collected from weeds in hedge rows and field margins during summer in Scotland were infective with the same yellow dwarf virus on winter barley the following season. However, no test was conducted on the grasses to confirm the virus presence.

In the 2 B/CYDV transmission studies reported here, winged *R. padi* did not survive after 3 d of feeding on bahiagrass. *Rhopalosiphum padi* is a known aphid vector of B/CYDV, and it has been associated with BYDV infection to wheat (Chapin et al. 2001). For bahiagrass to act as a source of inoculum in yellow dwarf epidemiology, aphid species other than *R. padi* will have to transmit the virus from bahiagrass to wheat.

Sipha flava or yellow sugarcane aphid was the only aphid that we found on bahiagrass in 2007 and 2008. Yellow sugarcane aphid is acknowledged as one of the 2 major aphid pests of pasture grasses in Florida (Sprenkel 2007). *Schizaphis graminum* (Rondani), the other prominent aphid pest of pasture grasses in Florida, was not found in the 2 years of our sampling of the 3 warm-season grasses. Among warm season grasses, *Sipha flava* is reported to multiply best on Japanese millet, *Echinochloa crusgalli frumentacea* (Link) W.F. Wight, and laurisa-grass, *Pennisetum orientale* (Wild) L.C. Rich (Kindler and Dalrymple 1999). In a host range test, the number of surviving *S. flava* after 2 wks of feeding on bahiagrass was lower than the initial colony, indicating that bahiagrass is not an optimum host for the aphid (Kindler and Dalrymple 1999). Apart from pasture grasses, *S. flava* also is known as a pest of sorghum, *Sorghum bicolor* (L.) Moench, and sugarcane, *Saccharum officinarum* L. (Blackman and Eastop 2000). A survey of wheat aphids in Alabama and western Florida showed that *S. flava* is usually found on winter wheat early in the season (Hadi et al. 2011).

A list of B/CYDV vectors compiled by Halbert and Voegtlin (1995) does not include *S. flava*. However, Blackman et al. (1990) commented that no study has been conducted to investigate the potential of *S. flava* as B/CYDV vector. To our knowledge, *S. flava* has not been tested for B/CYDV transmission.

Because our infectivity assays conducted on yellow sugarcane aphid collected in bahiagrass might have been negatively affected by accidental injuries associated with the sampling technique, we cannot conclude whether yellow sugarcane aphid can transmit yellow dwarf viruses. No known vector of yellow dwarf viruses was found on bahiagrass or limpograss in the 2 sampling years even though B/CYDV was found on both plant species. The aphid responsible for the introduction of B/CYDV into the 2 grasses is still unclear.

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