Barley Yellow Dwarf Luteoviruses and Their Predominant Aphid Vectors in Winter Wheat Grown in South Carolina

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ABSTRACT

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Barley yellow dwarf is recognized as an important disease problem in winter wheat production in the southeastern United States, but there is relatively little known about the ecology and epidemiology of barley yellow dwarf virus (BYDV) in this region. From 1991 to 1993, and in 1996 and 1997, winter wheat was sampled for BYDV throughout the principal wheat production areas in South Carolina. In addition, in 1997, a small number of samples were collected from fields in North Carolina and Kentucky. Plant samples were assayed to determine the BYDV serotype and, subsequently, coat protein sequences of isolates within the same serotype were compared using restriction fragment length polymorphisms. Representative BYDV isolates from South Carolina and type isolates from New York were compared in aphid transmission experiments using aphid species collected from South Carolina and laboratory colonies maintained in New York. The predominant BYDV serotype in South Carolina (in all years) was PAV, accounting for 94% of the total BYDV-infected samples analyzed. The RPV serotypes were more abundant in samples collected from western North Carolina and Kentucky. PAV isolates from all regions were identical to the New York BYDV-PAV in terms of serology and restriction fragment patterns. Furthermore, the aphid transmission phenotypes were similar for South Carolina and New York BYDV isolates. The predominant aphids colonizing winter wheat in South Carolina included *Schizaphis graminum*, *Rhopalosiphum rufiabdominalis*, *R. padi*, and *Sitobion avenae*. The South Carolina clones of *R. padi* and *S. avenae* were similar to the New York laboratory clones in their abilities to transmit various BYDV isolates from New York and South Carolina. In contrast to the New York clone of *Schizaphis graminum* that can vector SGV, PAV, and RPV, the *S. graminum* clone from South Carolina was not a vector of any BYDV serotype tested. *R. rufiabdominalis* was found to be an efficient vector of PAV, RPV, and RMV isolates, but did not transmit MAV or SGV.

Barley yellow dwarf viruses (BYDV) have caused sporadic, major disease epidemics in cereals grown in the southeastern United States for several decades (26,31). In more recent years, concurrent with the expanding winter wheat and winter oat acreage in the southeast, barley yellow dwarf (BYD) incidence has been increasing (2,7). BYDV has been reported from several southeastern states (7,10,20,34). PAV serotypes tend to predominate, although RPV serotypes are also common. MAV serotypes have not been reported and serological testing has not generally been done for RMV and SGV serotypes. Rochow (26) did identify BYDV isolates

collected in Florida that were transmitted by *Rhopalosiphum maidis* (Fitch), *Schizaphis graminum* (Rondani), or both, which suggests that RMV or SGV serotypes may be present in the southeast.

Estimates of BYD incidence in the southeastern United States vary widely between years, but the limited information indicates it is a serious problem in many years and grain yields can be significantly reduced (18,22). Use of insecticides to reduce the aphid populations and adjustment of fall planting dates to avoid the fall aphid flights are the recommended BYD control strategies in the region (D. Herselman, *personal communication*). Insecticidal control of aphids can decrease BYD incidence and increase yield (22). There is little information on the epidemiology of BYD in the southeast and the predominant aphid vectors of BYDV have not been identified. Without this information, insecticide applications and other control measures may be poorly timed and marginally effective.

Surveys of cereal aphid populations in South Carolina (J. Chapin, *unpublished*)

indicate that *S. graminum* and *R. rufiabdominalis* (Walker) colonize newly emerging plants in the fall, but generally only low population levels are present in the spring. *R. padi* (L.) also colonizes plants later in the fall, but it tends to overwinter on the wheat and significant populations can rapidly develop in the spring. *Sitobion avenae* (Fab.) usually does not develop significant populations until late in the growing season. Similar trends, especially for *R. padi* and *S. avenae*, have been reported from Virginia and Kentucky (18,21,22). Since the severity of BYD effects on plant growth and yield are positively correlated with the age of the plant when infected, fall aphid flights and fall infections are considered to be more significant in the epidemiology of the disease and yield loss on winter cereals (12,23,32).

All of the cereal aphids reported to colonize winter wheat in the southeastern United States are known to transmit BYDV (15); however, transmission efficiencies vary among clones within a species (14,24). Furthermore, the BYDV isolate-aphid species relationships are for the most part based on laboratory-maintained virus isolates and aphids, neither of which represent the range of virus isolates and the aphid clonal diversity that occurs in the field (24). Consequently, it is risky to make assumptions regarding what species of aphids are the vectors of various BYDV isolates. This statement is supported by the numerous examples of BYDV isolates that do not conform to the general vector-specific associations implied by the current BYDV classification system (24). The purpose of this study was to identify and characterize the predominate BYDV serotypes infecting the South Carolina winter wheat crop and to identify the aphid species that commonly colonize the crop and are the most likely candidates for transmitting the viruses within the crop.

MATERIALS AND METHODS

Aphid clones and virus isolates. The clones of the aphids maintained in New York include *S. avenae* (NY-Sa), *R. padi* (NY-Rp), *R. maidis* (NY-Rm), *Schizaphis graminum* (NY-Sg), and *Metopolophium dirhodum* (NY-Md). The history of NY-Sa, NY-Rp, NY-Rm, and NY-Sg were previously described (24). The *M. dirhodum* clone was collected in California by F. E.

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Gildow (11) and has been maintained in New York since 1982. In addition, aphids were collected in South Carolina from winter wheat plants at the Edisto Research and Education Center, Blackville, South Carolina between November 1995 and February 1996. Populations of *Sitobion avenae* (SC-Sa), *R. padi* (SC-Rp), *R. rufiabdominalis* (SC-Rr), and *Schizaphis graminum* (SC-Sg) were established as laboratory colonies in New York. Unlike the New York aphid populations, which were propagated from a single aphid and represent one clone, the South Carolina populations were started from multiple individuals and may represent more than one clonal population. The rearing conditions used for all aphids were described previously (28).

The BYDV isolates used include the previously characterized New York BYDV isolates referred to as NY-PAV, NY-MAV, NY-SGV, NY-RPV, and NY-RMV (28,30). Additional isolates were collected from infected winter wheat plants in South Carolina and are described below. All isolates were maintained in the greenhouse in New York in 'Coast Black' oat (*Avena byzantina* K. Koch) as described (28).

Field sampling. In 1991, flag leaves were collected from three symptomatic and three asymptomatic plants in 42 fields (3 to 6 fields per county) from 11 South Carolina coastal plain counties (Allendale, Bamberg, Barnwell, Calhoun, Clarendon, Darlington, Dillon, Florence, Lee, Orangeburg, and Sumter). The area surveyed encompassed the major wheat-producing counties within the state and extended from the North Carolina to Georgia borders. Fields were selected by following a predetermined route and arbitrarily deciding to sample the next field encountered. An effort was made to sample fields from widely divergent places within a county. In 1992 and 1993, three symptomatic flag leaves were taken from 33 fields in the same 11 counties (3 fields per county), for a total of 108 fields sampled over the 1991 to 1993 interval. Survey dates were 22 to 24 April in 1991 and 1992, and 26 to 29 April in 1993. Field maturity ranged from Zadocks GS 55 to 70, but most fields were between GS 65 and 70. An additional 143

symptomatic and 205 asymptomatic single-flag-leaf samples were collected from nine aphid management tests conducted in Aiken and Barnwell counties over the same 1991 to 1993 interval. All leaf samples were placed in paper envelopes in the field. The envelopes were kept in a plastic bag within an ice chest for return to the lab. Samples were refrigerated (4°C) for up to 7 days prior to shipment for serological assay. All enzyme-linked immunosorbent assays (ELISA) in 1991 to 1993 were conducted by AGDIA Inc. (Elkhart, IN).

In 1996, tillers expressing typical BYDV symptoms were randomly collected from two fields on the Edisto Research and Education Center in Barnwell County on 8 April. In addition, on 13 to 14 May, five symptomatic tillers were collected from each of several fields in eight coastal plain counties. Samples were shipped to New York and tested by ELISA. No attempt was made in 1996 to determine relative incidence of BYDV in each field or county; the purpose was only to identify which isolates were prevalent.

In 1997, one field on the Edisto Research and Education Center was sampled on 3 April and three grower fields were sampled on 15 and 16 April within each of 10 counties. Five symptomatic tillers were collected from each of the growers' fields to repeat the sampling scheme of 1996. To determine virus incidence and the frequency of non-symptomatic infections, an additional 25 tillers were randomly collected at each site. A total of 25 symptomatic and 100 non-symptomatic tillers were collected from a field on the Edisto Research and Education Center. Samples were shipped to New York and tested by ELISA. Symptomatic tillers were tested individually and non-symptomatic tillers were tested as composite samples of a portion of the leaves from five tillers. The remaining leaf tissue was saved and any composite samples testing positive were re-tested using leaf tissue from individual tillers.

Wheat samples were also collected by cooperators in North Carolina and Kentucky and shipped fresh to New York. Multiple tillers were randomly collected from test plots in Rowan and Davie coun-

ties in western North Carolina, and also from test plots at the Kinston Field Station in eastern North Carolina. Multiple composite samples of five tillers were tested by ELISA. Leaves showing typical BYDV symptoms were collected from fields in five Kentucky counties: Shelby, Caldwell, Christian, Schochoh, and Warren. Individual leaves from the Kentucky locations were tested by ELISA.

Virus transmission studies. Transmission of the New York and South Carolina BYDV isolates by the New York or South Carolina aphid clones was determined by allowing aphids a 24- to 36-h acquisition access period (AAP) on detached leaves from plants that had been tested for BYDV infection by ELISA. Aphids were transferred to 'Coast Black' oat seedlings (10 aphids per plant) and allowed a 72- to 96-h inoculation access period prior to fumigation. First through third instar *S. graminum* or *R. maidis* nymphs were used for the transmission assays, whereas a mixture of apterous adults and nymphal stages were used for all other aphid species.

Serological and polymerase chain reaction assays. Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) or triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) was performed as described (6,13). The polyclonal antibodies to the five New York-BYDV type isolates were described previously (29,35). The monoclonal antibodies RPV1, RPV3, and MAV4 have been described (17), as has PAV 1C2 (8). The absorbance values for healthy controls were <0.02 and samples were not considered infected unless the absorbance value was >0.1.

Nucleic acid extraction, reverse transcriptase polymerase chain reactions (RT-PCR), and subsequent restriction enzyme digests were performed as described (25) with the following modifications. Complementary DNA was synthesized and amplified in a 25- μ l reaction using the Titan One Tube RT-PCR System (Boehringer Mannheim, Germany) and the primers Lu1 and Lu4. The RT-PCR products were precipitated by adding sodium acetate to 0.3 M and 2.5 volumes of ethanol. The pellets were washed with 70%

Table 1. Barley yellow dwarf isolates detected in symptomatic and asymptomatic wheat leaves collected from South Carolina coastal plain counties, 1991 to 1993

Sample source	No. samples ^a	No. negatives	No. samples positive by isolate								
			PAV	MAV	RPV ^b	RMV	SGV ^b	PAV + MAV	PAV + SGV	RPV + SGV	RPV + RMV
County survey ^c	108 SY	5	101	0	0	0	0	1	1	0	0
	42 ASY	36	3	3	...	0	...	0	...	...	...
Field experiments ^d	143 SY	33	105	0	0	0	0	2	2	1	0
	205 ASY	196	4	0	1	0	3	0	0	0	1
Total	251 SY	38	206	0	0	0	0	3	3	1	0
	247 ASY	232	7	3	1	0	3	0	0	0	1

^a SY = symptomatic, ASY = asymptomatic.

^b ... = not tested for RPV and SGV in 42 symptomatic and asymptomatic samples from 1991 survey fields.

^c Eleven-county survey of 108 fields.

^d Nine field experiments in Aiken and Barnwell Counties.

ethanol, vacuum-dried, and resuspended in 25 µl sterile water. Aliquots of the resuspended RT-PCR product were digested with *Hae*III, precipitated as described above, washed with 70% ethanol, and analyzed on a 10% polyacrylamide gel.

RESULTS

BYDV isolate survey 1991 to 1993. PAV was the predominant isolate identified in the 1991 to 1993 surveys of growers' fields and in the Aiken and Barnwell county test plots (Table 1). Of the positive ELISA samples, 93% (213/228) contained PAV alone. MAV and SGV strains were each detected in approximately 3% of positive samples, either alone or in combination with PAV. RPV was detected in two

samples, and RMV in only one sample (combined with SGV). Of the symptomatic leaf samples, 38/251 (15%) were negative for all BYDV isolates, and 15/247 (6%) of asymptomatic samples tested positive by ELISA.

BYDV isolate identification and characterization in 1996. Of the 22 symptomatic samples analyzed from one field on the Edisto Research and Education Center, 16 samples tested positive for PAV, 4 tested positive for RPV, and 2 did not react with any of the five BYDV antisera. Of symptomatic samples from a second field, 14 tested positive for PAV, 6 for RPV, and 1 did not react with any of the BYDV antisera. No mixed infections of PAV and RPV were identified from either field. All PAV-positive samples, including the PAV-infected control, also cross-reacted with an anti-MAV polyclonal antibody and reacted with the PAV-specific monoclonal antibody, 1C2, but none reacted with the MAV-specific monoclonal antibody, MAV4. Nine of the PAV-positive samples were analyzed by RT-PCR and the product digested with *Hae*III. The restriction pattern for all the samples were identical and matched the pattern of NY-PAV. Figure 1A illustrates the restriction pattern for the NY-PAV and a representative PAV isolate from South Carolina. Virus from two of the PAV-positive leaf samples, one from each field, was separately transferred to 'Coast Black' oat seedlings using NY-Rp. These two isolates (SC-PAV 1-9 and SC-PAV 2-9) were used in subsequent transmission experiments discussed below. The RPV-positive samples did not react with any of the other BYDV polyclonal antisera tested, but all reacted with the RPV-specific monoclonal antibodies RPV1 and RPV3. Virus from three RPV-positive leaf samples was transferred to 'Coast Black' oat seedlings for use in subsequent experiments. The isolates were referred to as SC-RPV 1-16, SC-RPV 1-19, and SC-RPV 2-11. The coat protein gene of the three South Carolina RPV isolates was amplified by RT-PCR, and digestion of the 540-nucleotide coat

protein fragment by *Hae*III produced an identical pattern to the NY-RPV (*data not shown*).

PAV was detected from symptomatic leaves collected from commercial fields in each of the eight counties sampled, but RPV was found in only one of the eight counties (Table 2). All samples identified as PAV-infected reacted with the PAV-specific monoclonal antibody, 1C2, but not with the MAV-specific monoclonal antibody, MAV4. Furthermore, the *Hae*III digestion pattern of the coat protein nucleotide sequence amplified by RT-PCR was identical among all the PAV-infected samples and identical to the NY-PAV. The restriction pattern is different from other PAV serotypes (e.g., PAV129) commonly found in New York. The RPV samples all reacted with the monoclonal antibodies RPV1 and RPV3. The samples were not analyzed by RT-PCR.

Virus isolate survey and disease incidence in 1997. As in 1996, PAV was the predominant serotype and was detected in 89% of the symptomatic tillers (Table 3). RPV was present in 2% of the symptomatic plants and the other three serotypes, MAV, SGV, and RMV, were not detected. BYDV incidence in the randomly collected tillers was low in all fields and ranged from 0 to 7% (Table 3). Leaf tissue from one sample testing positive for PAV from each county was randomly selected and transferred using the NY-Rp to a 'Coast Black' oat seedling. Subsequently, the infected oat tissue was used for RT-PCR and restriction fragment length polymorphism (RFLP) analysis. Restriction enzyme digestion of all of the PAV RT-PCR products examined indicated that all were similar to each other and to NY-PAV (*data not shown*).

RPV and PAV were also the only BYDV serotypes detected in samples from North Carolina and Kentucky. PAV was more predominant in the eastern sampling locations of North Carolina and Kentucky, whereas RPV was more prevalent in western North Carolina and central Kentucky.

Fig. 1. Restriction fragment length polymorphisms of polymerase chain reaction (PCR) products from partial coat protein gene sequences of representative barley yellow dwarf virus (BYDV) PAV and RPV isolates. All PCR products were digested with *Hae*III. (A) BYDV isolates from New York include the PAV and RPV type isolates, and PAV-129, an isolate commonly found infecting winter wheat in New York is shown for comparison as a PAV isolate with a different restriction pattern. PAV129 produces severe symptoms in oat (6). Also, PAV isolates obtained from winter wheat collected in South Carolina, North Carolina, and Kentucky. (B) The New York RPV isolate and two RPV isolates obtained from winter wheat from Kentucky. KY-1 RPV is representative of all isolates analyzed from Kentucky, with the exception of isolate KY-2 RPV. An undigested (uncut) PCR product is shown for reference.

Table 2. Barley yellow dwarf virus (BYDV) isolates detected by enzyme-linked immunosorbent assay in symptomatic wheat leaves collected at various locations in the major wheat-growing counties in South Carolina

County ^b	No. fields sampled	BYDV serotype ^a	
		PAV	RPV
Edisto Field Station, Barnwell	2	2	2
Clarendon	2	1	0
Darlington	5	3	0
Dillon	1	1	0
Florence	4	1	0
Lee	4	3	0
Marion	1	1	0
Orangeburg	3	3	0
Sumter	4	4	2

^a Number of fields in which the BYDV serotype was identified in any of the wheat samples. MAV, SGV, and RMV were not detected in any samples.

^b Samples were collected on 8 April 1996, at the Edisto Field Station; samples from commercial fields in the other eight counties were collected on 13 to 14 May 1996.

PAV was detected in 3 of 24 non-symptomatic, composite samples from eastern North Carolina, whereas samples from western North Carolina were infected with PAV and RPV; in 10 Rowan County samples, 1 was RPV + PAV, 1 was PAV, and 5 were RPV; in 60 Davie County samples, 21 were RPV + PAV, 8 were PAV, and 15 were RPV. Samples from southeastern Kentucky were infected predominantly by PAV; in 5 Schochoh County samples, 4 were PAV and 1 was PAV + RPV. PAV was also found in 4 of 5 symptomatic plants from Shelby County in north central Kentucky. Samples from central Kentucky were infected predominantly by RPV; in 8 Caldwell County samples, 7 were RPV and 1 was RPV + PAV; in 8

Christian County samples, 6 were RPV and 2 were PAV.

The *Hae*III digestion pattern of the PAV coat protein nucleotide sequence amplified by RT-PCR was identical for all samples sent from North Carolina and Kentucky and to the NY-PAV (Fig. 1A). The RFLP analysis of the RPV coat protein sequences indicated that all isolates from North Carolina and Kentucky were similar to each other and the NY-RPV with the exception of one RPV isolate from central Kentucky (Fig. 1B).

Comparative transmission of the New York BYDVs by New York and South Carolina aphids. The four South Carolina aphid species were tested for their ability to transmit the five New York BYDV iso-

lates in multiple experiments and the results compared to transmission of the same isolates by the New York clones of the aphids (Table 4). *R. rufiabdominalis* has not been identified in New York. SC-Rr was an efficient vector of NY-PAV, NY-RPV, and NY-RMV in three independent experiments. The SC-Rp was similar to the NY-Rp in that it efficiently transmitted NY-PAV and NY-RPV and did not transmit NY-MAV or NY-SGV. However, the SC-Rp did efficiently transmit NY-RMV, whereas the NY-Rp did not. The SC-Sa and NY-Sa were similar in their efficient transmission of NY-PAV and NY-MAV and their inability to transmit NY-SGV. However they differed significantly in that the SC-Sa was able to transmit the NY-RPV and NY-RMV more efficiently than the NY-Sa. The NY-Sg, which originally came from Wisconsin (24), is an efficient vector of NY-SGV and consistently transmits NY-PAV, NY-RPV, and, to a lesser extent, NY-RMV. In contrast, the SC-Sg did not efficiently transmit any of the five isolates.

Both the NY-Sg and SC-Sg appeared to feed well on the BYDV-infected oat tissue and there was no obvious behavioral differences on the recipient oat plants. To determine if both clones were acquiring virus, aphids were allowed a 48- to 72-h AAP on leaves infected with NY-PAV or NY-SGV. Groups of 25 aphids were then tested by ELISA. Groups of 10 aphids from each treatment were also transferred to four 'Coast Black' oat seedlings to test for transmission. PAV and SGV antigen was detected in both the NY-Sg and the SC-Sg clones. The mean SGV ELISA values (A_{405}) were 0.241 ($n = 8$) and 0.241 ($n = 8$) for the NY-Sg and SC-Sg, respectively. The mean PAV ELISA values (A_{405}) were 0.139 ($n = 6$) and 0.281 ($n = 6$) for the NY-Sg and SC-Sg, respectively. ELISA values of healthy controls were <0.02 and <0.01 for SGV and PAV, respectively. The transmission was consistent with earlier experiments; NY-Sg transmitted SGV and PAV to 75 and 50% of the plants, respec-

Table 3. Barley yellow dwarf virus (BYDV) serotypes identified from plants expressing typical barley yellow dwarf disease symptoms (symptomatic) or from plants randomly collected

County	Sample type	BYDV serotype ^a		
		PAV	PAV + RPV	RPV
Edisto Field Station	Symptomatic	24/25	0/25	1/25
	Random	7/100	0/100	1/100
Barnwell	Symptomatic	12/15	1/15	0/25
	Random	2/75	0/75	0/75
Allendale	Symptomatic	13/15	0/15	1/15
	Random	1/75	0/75	1/75
Bamberg	Symptomatic	15/15	0/15	0/15
	Random	0/75	0/75	0/75
Orangeburg ^b	Symptomatic	7/10	0/10	1/10
	Random	1/75	0/75	0/75
Calhoun	Symptomatic	13/15	0/15	0/15
	Random	1/75	0/75	0/75
Clarendon	Symptomatic	14/15	0/15	0/15
	Random	0/75	0/75	0/75
Lee	Symptomatic	14/15	0/15	1/15
	Random	2/75	0/75	0/75
Darlington	Symptomatic	15/15	0/15	0/15
	Random	1/75	0/75	0/75
Dillon	Symptomatic	11/15	0/15	0/15
	Random	0/75	0/75	0/75
Florence	Symptomatic	3/15	0/15	0/15
	Random	0/75	0/75	0/75

^a Number of plants testing positive by enzyme-linked immunosorbent assay/total number of plants tested. Three fields were sampled in each county. A total of 25 plants (symptomatic or asymptomatic) were randomly collected in addition to 5 symptomatic plants from each field. The exception was a collection from the Edisto Field Station that consisted of 25 symptomatic plants and 100 randomly selected plants.

^b No symptomatic samples were collected from one of the three fields in this county.

Table 4. Transmission of five New York (NY) isolates of barley yellow dwarf virus (BYDV) by four aphid species collected in South Carolina in 1996 and the comparable transmission by clones of the aphids maintained in New York for 30+ years

Isolate	South Carolina aphid clones ^a								New York aphid clones ^a					
	Rh-pa		Rh-ru		Si-av		Sc-gr		Rh-pa		Si-av		Sc-gr	
	Total ^b	Mean (%) ^{c,d}	Total	Mean (%)	Total	Mean (%)	Total	Mean (%)	Total	Mean (%)	Total	Mean (%)	Total	Mean (%)
NY-PAV	23	100	36	100	22	100	32	3*	753	99	612	79	576	36
NY-RPV	23	100	36	100	56	18**	32	3*	896	99	680	2	613	37
NY-RMV	36	73**	36	100	36	13**	36	16	634	10	630	2	616	16
NY-MAV	24	0	36	0	24	100	36	0	669	2	913	99	631	1
NY-SGV	24	0	36	0	33	0	44	3**	456	0	455	1	632	88
Healthy	12	0	16	0	12	0	20	0						

^a Aphids used: Rh-pa = *Rhopalosiphum padi*, Rh-ru = *R. rufiabdominalis*, Si-av = *Sitobion avenae*, and Sc-gr = *Schizaphis graminum*. *R. rufiabdominalis* does not occur in NY.

^b Total number of recipient plants used in all experiments. Each aphid-virus combination was tested in 3 to 5 independent experiments.

^c Transmission was calculated as the number of infested plants (10 aphids/plant) that became infected, means were calculated from $N = 3$ to 5 independent experiments. A Chi-square test was calculated to compare the difference between mean transmission efficiencies of the NY-BYDV isolates by the South Carolina and New York aphid biotypes. Significant differences: ** and * indicate $P = 0.01$ and 0.05 , respectively.

^d The mean transmission was calculated from numerous experiments conducted over the past 25 to 35 years (24).

tively. The SC-Sg did not transmit either SGV or PAV.

Comparative transmission of the South Carolina BYDVs by New York and South Carolina aphids. Three isolates of PAV were tested. One (F-22) was obtained from a viruliferous *R. padi* alate in November, 1995; and isolates 1-9 and 2-9 were obtained from infected leaves collected from two different fields at the Edisto Research and Education Center in May 1996. Three isolates of RPV were tested; all were obtained from infected leaves collected at the Edisto Research and Education Center in May 1996. Mean transmission data are presented in Table 5 for the PAV isolates ($n = 2$ to 4) and RPV isolates ($n = 2$). There were no obvious differences in the transmission phenotypes among either the SC-PAV or SC-RPV isolates. In addition, the symptom severity on 'Coast Black' oat was similar for all of the South Carolina isolates.

As was observed for transmission of the New York BYDVs, both the NY-Rp and SC-Rp transmitted the SC-PAVs and SC-RPVs efficiently. The NY-Sa and SC-Sa both transmitted the PAVs efficiently, but transmitted the RPVs inefficiently. Transmission by the NY-Sg varied among the three experiments, but the range of mean transmission of 33 to 52% and 50 to 56% for the SC-PAVs and SC-RPVs, respectively, was similar to that for the NY-PAV (36%) and NY-RPV (37%) (24). In contrast, the SC-Sg did not transmit any of the RPV isolates or the SC-PAV F-22 isolate and transmitted the other two SC-PAVs inefficiently. The SC-Rr transmitted all of the PAVs and RPVs efficiently, whereas the NY-Rm and NY-Md were either unable to transmit or transmitted poorly.

DISCUSSION

Field surveys support previous findings that, in the southeastern United States, the predominant BYDVs in the winter wheat crop belong to the PAV serotype, although RPV serotypes are also commonly found (7,20,34). A majority of the infections in our surveys were symptomatic. The few non-symptomatic infections were the same isolates found in symptomatic infections

and were likely the result of infections that occurred later in the growing season. The symptomatic samples that did not test positive for any of the BYDV serotypes were not tested further. They may have contained an unidentified BYDV serotype, but more likely the general chlorosis was the result of another disease or nutrient deficiencies.

The virus isolates that were identified and characterized in the field surveys were similar to each other by ELISA, RFLP analysis of the coat protein gene, aphid transmission phenotype, and symptom severity. This is in contrast to PAV isolates in New York, where considerable serological and nucleotide sequence diversity, as well as varying degrees of virulence in oat genotypes, are observed (6). Considerable diversity of PAV isolates has also been reported from Europe (4,5). Diversity among RPV isolates has not been examined in the United States; however, differences in virulence among RPV isolates was reported from Australia (1). There are also several reports describing the diversity of isolates within other BYDV serotypes on a wider geographical scale (3,9,19). The above-mentioned studies suggest that diversity among BYDV isolates in any given serotype is common. The homogeneity of the PAV and RPV isolates identified from South Carolina, North Carolina, and Kentucky suggest a relatively stable population of virus and vectors in the region. Immigration of new biotypes of vectors or new virus isolates may not be common within the southeastern United States. However, the finding of one RPV isolate from Kentucky that had a different restriction pattern indicates that variability does exist; additional testing is needed to define the extent of the diversity and determine whether there is any biological significance to such traits.

Our transmission data provides indirect information on the relative importance of the various cereal aphids in the epidemiology of BYDV in South Carolina. Although *S. graminum* is commonly found migrating into and colonizing the newly emerging South Carolina wheat crop, the lack of transmission of any BYDV isolates by the

SC-Sg suggests that this aphid does not play a major role in BYDV transmission. Rochow (27) reported efficient BYDV transmission by *S. graminum* biotypes from Wisconsin and Illinois, but a biotype from Florida was an inefficient vector of BYDV. Perhaps *S. graminum* is anholocyclic in the southeast and the population is homogeneous, in contrast to the *S. graminum* populations in the midwestern and western United States (33). SGV was detected in few wheat samples analyzed in this study, which provides additional indirect evidence that *S. graminum* is not a primary BYDV vector in the southeastern United States. SGV serotypes and *S. graminum* are common from Texas northward through the plains states and into the northwestern United States (16,19). We were able to document that, although the SC-Sg was unable to transmit any of the BYDVs, the aphids were able to ingest amounts of NY-SGV and NY-PAV similar to NY-Sg. These two *S. graminum* clones may provide an interesting experimental system to further investigate the vector specific transmission of BYDV.

The SC-Rr population was an efficient vector of the PAV and RPV isolates found in South Carolina. This aphid can colonize wheat in appreciable numbers early in the growing season, but usually dies off as the temperatures drop. In contrast to the northwestern United States, spring populations of *R. rufiabdominalis* are not common in the southeast (15). This aphid may be responsible for some primary infections of the crop; however, additional studies are needed to determine the numbers of viruliferous *R. rufiabdominalis* that migrate into the emerging winter wheat.

Sitobion avenae is commonly found colonizing wheat in the southeastern United States in the spring and builds to high populations. We have found this aphid to be an efficient vector of the SC-PAV isolates and an inefficient vector of the SC-RPV isolates. This aphid may be involved in secondary spread of the virus within the crop during the spring, but it is unlikely that these late infections are responsible for much of the yield loss attributable to BYDV infection. However, the late-season

Table 5. Percent transmission of South Carolina isolates of barley yellow dwarf virus belonging to the PAV and RPV serotypes by aphids from New York (NY) and South Carolina (SC)

Aphid species	PAV Serotypes			RPV Serotypes		
	F-22	1-9	2-9	1-16	1-19	2-11
NY <i>Metopolophium dirhodum</i>	19 ± 27 ^a	26 ± 16	8 ± 12	0	0	0
NY <i>Rhopalosiphum maidis</i>	0	0	0	0	0	0
NY <i>R. padi</i>	100	100	100	100	88 ± 18	100
SC <i>R. padi</i>	100	100	100	100	100	100
NY <i>Sitobion avenae</i>	94 ± 9	100	63 ± 18	13 ± 18	0	13 ± 18
SC <i>S. avenae</i>	93 ± 10	100	94 ± 9	0	0	6 ± 9
NY <i>Schizaphis graminum</i>	42 ± 44	52 ± 46	33 ± 26	50 ± 18	56 ± 44	50 ± 0
SC <i>S. graminum</i>	0	4 ± 7	4 ± 7	0	0	0
SC <i>R. rufiabdominalis</i>	67 ± 38	74 ± 34	78 ± 30	100	100	100

^a Mean (± standard deviation) transmission efficiency calculated as the number of infected plants/inoculated plants × 100. A total of 10 aphids were transferred to each of 12 recipient plants; experiments were repeated two to four times.

infections may play a significant role in increasing overall disease incidence and consequently providing inoculum for infecting corn or perennial grasses that may serve as local overwintering hosts for the virus and aphids. Additional work to investigate BYDV infection of these potential reservoirs is warranted.

Our findings from the field and laboratory studies point to *R. padi* as the primary vector of BYDV in the South Carolina winter wheat crop. This aphid is an efficient vector of all of the BYDV isolates from South Carolina tested in this study. *R. padi* usually migrates into the crop in the fall and overwinters in the wheat. This can result in large, rapidly developing populations of *R. padi* early in the spring. Ongoing experiments are directed toward determining the most efficacious suppression strategies for this vector.

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