Barley Yellow Dwarf Viruses in Wheat, Endophyte-Infected and Endophyte-Free Tall Fescue, and Other Hosts in Arkansas

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ABSTRACT

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An indirect enzyme-linked immunosorbent assay was used to test for PAV, RPV, and MAV serotypes of barley yellow dwarf viruses (BYDVs) in symptomatic and randomly collected samples from commercial wheat plantings and in other hosts that may be important sources of BYDVs for infection of fall-planted wheat in Arkansas. In symptomatic wheat samples, PAV serotypes were predominant (97%), RPV serotypes were found occasionally (3%), and no MAV serotypes were found. The occurrence of the three serotypes was similar in randomly collected wheat samples. PAV serotypes were detected in johnsongrass, oat, rye, and tall fescue, which may serve as oversummering hosts for the virus. In tall fescue, PAV serotypes were detected in fewer plants infected with the endophyte *Acremonium coenophialum* than in endophyte-free tall fescue.

Barley yellow dwarf (BYD) is a damaging disease of small grains that occurs worldwide and is caused by a group of luteoviruses referred to as barley yellow dwarf viruses (BYDVs). BYDVs cause varying degrees of yellowing, reddening, purpling, stunting, and yield reduction in small grains and other gramineous plants. Rochow (18) characterized and designated five strains of BYDVs and gave each an abbreviation from the initial letters of its principal vector species. The three most studied BYDV strains are PAV, RPV, and MAV (19). The BYDVs are antigenically distinct, and the production of monoclonal antibodies specific for each of the BYDVs (9) has led to a more sensitive enzyme-linked immunosorbent assay (ELISA) for detecting their incidence in the field.

Soft red winter wheat (*Triticum aestivum* L.) production in Arkansas has increased from 215,000 ha in 1979 (1) to 486,000 ha in 1989 (2). Although symptoms characteristic of BYD in wheat in Arkansas have been noted over the last 10 yr, no systematic surveys have been conducted to determine the incidence of BYDVs in Arkansas. Several cereal crops and perennial grasses grown in Arkansas, both weedy and cultivated, are hosts of BYDVs (12,13).

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In Arkansas, there are approximately 800,000 ha of tall fescue (*Festuca arundinacea* Schreber; 8). It is planted as a major perennial forage grass and by the highway department as cover in median strips and roadside ditches. In Missouri, 60% of tall fescue samples collected around the state were infected with BYDVs (12). Tall fescue has been reported as a symptomless host of BYDVs (4,17). This grass is a host for the bird cherry oat aphid (*Rhopalosiphum padi* (L.); 16), a vector of PAV that is the most prevalent serotype of BYDV (21).

Most of the tall fescue growing in Arkansas is infected with *Acremonium coenophialum* Morgan-Jones & W. Gams, an endophyte that induces fescue toxicosis in grazing animals (8). Recently, endophyte-free fescue was introduced to avoid the problem of fescue toxicosis. However, the presence of the endophyte may deter feeding by different types of insects, including aphids (16). Thus, endophyte-free fescue might be readily used as a food source and colonized by aphid vectors of BYDVs.

The objectives of this study were to determine which BYDVs occur in cereal crops and perennial grasses in Arkansas and to determine incidence of BYDVs in tall fescue with or without the endophyte *A. coenophialum.*

MATERIALS AND METHODS

Statewide BYDV survey. Samples of symptomatic wheat were collected statewide from commercial plantings during April and May of 1989 and 1990. Plants were considered symptomatic if leaves had purple or yellow discoloration starting at the tip and proceeding toward the base. Samples were collected near Kibler and Fayetteville (northwest Arkansas); Pine Tree, Bald Knob, and Keiser (northeast Arkansas); and Foreman and Hope (southwest Arkansas). Four to five leaves from each of 42 to 84 plants were collected from each location for a total of 462 samples in 1989 and 294 in 1990. Three to four leaves from 84 plants were also collected without regard for symptoms, following a grid pattern with a 4-m spacing in 28 × 48 m blocks at Kibler, Pine Tree, and Foreman from March to May of 1989 and 1990 to estimate the incidence of infection. Samples were frozen at −20 C for 2-3 mo until assayed by ELISA for PAV, RPV, and MAV.

To survey for oversummering hosts, leaf samples of grasses were collected at Fayetteville, Hope, and Pine Tree from the borders surrounding wheat fields. Twenty-one to 84 samples were taken from what appeared to be the most prevalent grasses in the sampling area. Although the majority of the plants did not show symptoms, efforts were made to collect leaves from yellow, purple, or stunted plants. A total of 755 samples were collected and identified (by E. Smith, University of Arkansas) in the summer of 1989. Samples were assayed only for PAV, because the survey of BYDVs in the spring of 1989 indicated that PAV was the predominant BYDV type in wheat in Arkansas.

Samples also were collected from oat (*Avena sativa* L.), sorghum (*Sorghum bicolor* L.), corn (*Zea mays* L.), rice (*Oryza sativa* L.) and tall fescue. Samples were taken from fescue pastures in the southwestern part of the state that had been established for at least 3 yr and that were infected with the endophyte or were endophyte-free. Samples from the crops were collected without regard for symptoms except for oat, which displayed typical BYD symptoms. Samples were frozen and then tested for PAV and RPV.

BYDV survey from endophyte-infected and endophyte-free tall fescue. Plots of endophyte-free and endophyte-infected tall fescue cv. Kentucky 31 were established from seed in 1986 at the

University Farm at Fayetteville on a fine-loamy, siliceous soil. The experiment was laid out as a randomized block design with eight replications and two levels of endophyte infection, 0 and 75% in endophyte-free and endophyte-infected treatments, respectively. The level of endophyte infection was measured by randomly sampling 20 5-cm tiller bases in each plot in early June, 1987. Leaf sheath sections were stained and microscopically examined for endophyte (22). Infection was expressed as a percentage frequency of hyphal detection in the tiller samples. In the fall of 1989 and 1990, leaf samples for analysis of BYDVs were collected from 11 plants at random from each replication. For each sample, leaves were collected from a single plant and stored at −20 C. Data from both years were analyzed together, and year was treated as a split-plot factor.

In April of 1990, colonies of *R. padi* were collected from endophyte-free plots of tall fescue, and three or four aphids from each colony were placed on single Clintland-64 oat seedlings to assess incidence of BYDV transmission. After a 6-day inoculation access, aphids were destroyed by spraying with 0.5% malathion, and plants were maintained in the greenhouse for 2 wk. The oat seedlings were harvested and frozen prior to assays for PAV.

Serological analysis. Triple-antibody sandwich ELISA was used to detect PAV, RPV, and MAV in the samples, as described by D'Arcy et al (9). Positive controls for the three serotypes were air-dried infected barley or oat tissue, and negative controls were extracts from healthy plants. Samples were prepared either by grinding 0.5 g of frozen tissue with a mortar and pestle in 2.5 ml of extraction buffer (phosphate-buffered saline plus 0.05% Tween 20 and 0.1% nonfat dry milk), or by extracting approximately 0.4 g of frozen tissue in 2 ml of extraction buffer with a leaf press (Erich Pollahne Co., Wennigsen, Germany).

Microtiter plates were coated with rabbit polyclonal antisera to Illinois (IL) isolates of BYDV (MAV-IL, RPV-IL, and PAV-IL) or the polyclonal antiserum MAV 2B3 from Richard Lister (Purdue University, IN). The monoclonal antibodies PAV-IL-1, RPV-IL-1, MAV-IL-1 (C. J. D'Arcy; 9), or MAFF-1 (Ian Barker, Harpenden, U.K.) were used as detecting antibodies. In 1989, tests for MAV utilized the polyclonal antiserum MAV 2B3 for trapping and MAFF-1 for the detecting monoclonal antibody. In 1990, the polyclonal antibody MAV-IL and the monoclonal antibody MAV-IL-1 were used. The monoclonal antibodies were detected with a commercial alkaline phosphatase conjugate of rabbit antibody to mouse immunoglobulin, or in the case of MAFF-1, antibody to rat immunoglobulin made in rabbits (Sigma, St. Louis, MO). Each plate contained noninfected controls (six wells per plate), infected controls (four wells per plate), and paired replicates of 42 samples. Each sample was analyzed for PAV, RPV, and MAV, except for the fescue samples from the endophyte-free and endophyte-infected plots, which were tested only for PAV.

Absorbance values were read on a microtiter plate reader (MR 300, Dynatech Laboratories, Chantilly, VA) at 410 nm (A_{410}). Field samples were considered positive when the mean A_{410} value of the replicate samples was greater than two times the mean absorbance of negative controls in the same plate.

Table 1. Detection of barley yellow dwarf viruses PAV, RPV, and MAV by ELISA[a] in symptomatic wheat in Arkansas during 1989 and 1990

Year and location of collection	No. of samples	No. of plants positive for BYDV serotypes			Percent positive[b]
		PAV	RPV	MAV	
1989 Tests[c]					
Fayetteville	84	82	4	0	98
Foreman	42	42	1	0	100
Bald Knob	42	41	1	0	98
Hope	84	82	0	0	98
Pine Tree	42	42	1	0	100
Kibler	84	83	3	0	99
Keiser	84	80	2	0	98
1990 Tests[d]					
Fayetteville	84	84	6	0	100
Bald Knob	84	75	4	0	89
Kibler	84	75	5	0	90
Pine Tree	42	35	1	0	83

[a] Enzyme-linked immunosorbent assay.
[b] Plants were considered noninfected if the ELISA tests were negative for all three serotypes.
[c] Samples were collected during April and May 1989.
[d] Samples were collected during April and May of 1990.

Table 2. Detection of barley yellow dwarf viruses PAV, RPV, and MAV in Arkansas wheat by ELISA[a] from samples collected randomly without regard for symptoms

Year and location of collection	No. of samples	No. of plants positive for BYDV serotypes			Percent positive[b]
		PAV	RPV	MAV	
1989 Tests					
Kibler[c] (March)	84	2	4	0	7
Kibler[d] (May)	84	7	0	0	8
1990 Tests					
Kibler[c] (March)	84	0	0	0	0
Kibler[d] (May)	84	1	0	0	1
Pine Tree[c] (March)	84	7	0	0	8
Pine Tree[d] (May)	84	10	0	0	12
Foreman[c] (March)	84	6	0	0	7

[a] Enzyme-linked immunosorbent assay.
[b] Plants were considered noninfected if the ELISA tests were negative for all three serotypes.
[c] Samples were collected when plants were in the stem elongation stage.
[d] Samples were collected when the plants were in the soft dough stage.

RESULTS

Statewide BYDV survey of symptomatic wheat. In 1989 and 1990, PAV was most commonly detected in symptomatic wheat samples. MAV was not detected in any samples. Of 462 wheat samples tested in 1989, 454 were positive for BYDV (Table 1). PAV was detected in 452 of the 454 BYDV-positive samples, and 12 samples were positive for RPV. Except for two samples from Keiser that were infected with RPV singly, all samples positive for RPV also were positive for PAV.

Of the 294 wheat samples tested in 1990, 270 were positive for BYDVs. PAV was detected in 269 of 270 positive samples. RPV was detected in 16 samples, and no samples were positive for MAV. At all locations in 1990, some samples reacted positively for both PAV and RPV (Table 1).

Survey of BYDVs in wheat collected without regard for symptoms. Two of 84 samples selected in a grid pattern without regard for symptoms at Kibler in March of 1989 were positive for PAV, and four samples were positive for RPV (Table 2). Of the 84 samples selected at random from the same field area at Kibler in May, seven were positive for PAV, but all were negative for RPV.

In 1990, samples were collected in a grid pattern from Kibler, Pine Tree, and Foreman. Of the 84 samples collected from Kibler in March, none were positive for BYDVs, and only one of 84 samples collected in May was positive for PAV

(Table 2). None of the samples were positive for RPV or MAV. At Pine Tree, only PAV was detected, and there was an incidence of 8% in March versus 12% incidence detected in samples collected in May. Seven percent of the early season samples from Foreman were infected with PAV. A flood prevented late-season sampling at the Foreman location.

Survey of BYDVs in grasses and crops other than wheat. In the summer of 1989, the weeds *Sorghum halepense* (L.) Pers., *Setaria geniculata* (Lam.) P. Beauv., *Paspalum dilatatum* Poir., *Brachiaria platyphylla* (Griseb.) Nash, *Digitaria ciliaris* (Retz.) Koeler, *Cyperus echinatus* (Ell.) Wood, *Echinochloa muricata* (Beauv.), *E. crus-galli* (L.) P. Beauv., and *Eleusine indica* (L.) Gaertn. were surveyed for the presence of the PAV serotype. Of 755 samples from the above plants, only one of 147 samples collected of *S. halepense* was positive for PAV.

Of a total of 712 samples collected in 1989 and 1990 from *S. bicolor, A. sativa, S. cereale, Z. mays, O. sativa,* and *F. arundinacea,* 50 plants of four species were positive for the PAV serotype (Table 3). No samples were positive for RPV. Only 2% of fescue samples from pastures in southwestern Arkansas were positive for BYDVs.

Survey of BYDVs in endophyte-infected and endophyte-free tall fescue. Results from 1989 and 1990 were not significantly different, and data were averaged across years. The incidence of PAV infection was significantly different in endophyte-infected (36.4%) and endophyte-free (73.6%) tall fescue.

Transmission of BYDVs by aphids colonizing tall fescue. Numerous colonies of *R. padi* were noted on endophyte-free fescue in the plots at Fayetteville in the spring of 1990 but not during the fall and winter months of 1989. Colonies of *R. padi* from 19 of 20 endophyte-free plants of tall fescue in the spring of 1990 transmitted the PAV serotype of BYDV to Clintland-64 oat seedlings. Tests in the fall of 1989 indicated that 87% of the tall fescue plants in these plots were infected with the PAV serotype.

Table 3. Assays for barley yellow dwarf viruses PAV and RPV in samples of crops other than wheat[a]

Location Host[b]	No. infected with PAV/no. tested[c]
Fayetteville	
Sorghum bicolor[d]	0/63
Avena sativa[e]	38/42
Secale cereale[e]	6/42
Zea mays[d]	0/21
Hope	
Sorghum bicolor[d]	2/126
Festuca arundinacea[d]	4/344
Bald Knob	
Oryza sativa[e]	0/84

[a] RPV was not detected in any samples.
[b] Except for *A. sativa*, all samples were randomly collected without regard for symptoms.
[c] Plants were considered noninfected if enzyme-linked immunosorbent assays were negative for PAV and RPV.
[d] Samples were collected in July and August of 1989.
[e] Samples were collected in June of 1990.

DISCUSSION

The sampling locations of Pine Tree, Kibler, and Foreman are located in important areas for wheat production in Arkansas. The PAV serotype is the most common BYDV in wheat and other crops in Arkansas, and as much as 8% of the wheat was infected by March following fall planting. The finding that PAV was the predominant serotype is similar to results from California (14), Indiana (7), Pennsylvania (11), and New York (20).

In our statewide survey of BYDVs in Arkansas, no sample was positive for MAV. Moreover, very few symptomatic plants were negative for both PAV and RPV. We conclude that PAV and RPV are responsible for most of the BYD symptoms seen in the field. However, RPV was present in only 4% (28/724) of the symptomatic wheat samples that were indexed positive for BYDVs.

Results of surveys for occurrence of BYDVs in wheat in fall and early spring indicated that symptoms are a poor indication of infection in young wheat (10). Fall infection has been reported to cause the most serious economic effect on winter wheat (5,6), but symptoms are very difficult to detect in young plants in the fall. For example, samples of very young wheat plants collected in the early fall of 1989 exhibited typical BYD symptoms in the field but were negative for BYDVs. These young wheat plants were colonized by the sugarcane aphid (*Melanaphis sacchari* (Zehntner)), which may have induced BYDV-like symptoms, or these plants may have been infected by a BYDV serotype other than those tested for in this study. In addition, symptomless young wheat collected in the fall of 1989 indexed positive for BYDV. The detection of BYDVs by ELISA in wheat suggests that symptoms are not a dependable diagnostic test in young wheat but that diagnosis based on symptoms is reliable in wheat after heading.

Most summer crops and weedy grasses apparently are not important oversummering hosts of BYDVs in Arkansas. Only one of 21 samples of *S. halepense* (johnsongrass) from Fayetteville was positive for PAV. Tall fescue may be an important oversummering host for BYDV. The difference in the incidence of PAV in fescue in Fayetteville and in southern Arkansas may be due to differences in cultural practices. The research plots at Fayetteville were mowed every 4 wk throughout the spring, summer, and fall for 3 yr, which may have redistributed viruliferous aphids (especially in the spring, when numerous *R. padi* were present on fescue). The differences also may be due to differences in climate between northwestern Arkansas and southern Arkansas. Fayetteville data on BYDV were similar to results obtained in Missouri, where the incidence of BYDV in tall fescue was found to be 59.6% (12). The endophyte status and age of the fescue plants in that study were not reported.

This is the first report of a negative association between BYDV infection and the incidence of *A. coenophialum* in tall fescue. Guy (15), on the other hand, recently reported that there was no correlation, positive or negative, between BYDV infection and the incidence of the perennial ryegrass endophyte *Acremonium lolii* Latch, Christensen & Samuels in perennial ryegrass (*Lolium perenne* L.). The lack of an effect of endophyte on BYDV infection in perennial ryegrass may be explained by the findings of Latch et al (16), who reported that *R. padi* showed no preference for *A. lolii*-infected or uninfected ryegrass, but that *R. padi* moved from *A. coenophialum*-infected to uninfected tall fescue plants.

Since various BYDVs have different effects on commercial cereal cultivars, a knowledge of the BYDVs present in wheat in Arkansas should be helpful for developing breeding strategies. Greenhouse yield-loss trials conducted on the wheat cultivar Anza in California (14) showed that it was tolerant to both PAV and MAV but not to RPV. Barley accessions with the Yd_2 gene have been described as resistant to a PAV isolate but not to an RPV isolate (3). On the basis of our finding that PAV was the most prevalent BYDV in Arkansas, future control of BYDV in Arkansas may utilize tolerance, such as that described in Anza wheat, or resistance through the incorporation into wheat of the Yd_2 gene from barley.

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