Characterization of a Nonspecifically Aphid-Transmitted CA-RPV Isolate of Barley Yellow Dwarf Virus

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ABSTRACT

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An isolate (CA-RPV-1) of barley yellow dwarf virus (BYDV) recovered from barley (*Hordeum vulgare*) in Davis, CA, was transmitted to oats (*Avena sativa*) in a nonspecific manner by New York (NY) clones of *Rhopalosiphum padi, Sitobion avenae*, and *Schizaphis graminum* and by a California (CA) clone of *Sitobion avenae*. The oat plants showed characteristic BYDV symptoms. In double-antibody-sandwich and indirect monoclonal antibody ELISA, CA-RPV-1 reacted strongly to antisera made to NY-RPV and in some tests to a lesser degree to antisera made to NY-PAV or NY-MAV. CA-RPV-1 was serologically indistinguishable from NY-RPV, regardless of which aphid species had transmitted it. The dsRNA banding pattern from plants infected by CA-RPV-1 was identical to that of NY-RPV. CA-RPV-1 did not react with recombinant cDNA plasmids made to NY-RPV, NY-PAV, NY-MAV, Australian PAV isolates of BYDV, or beet western yellows virus in nucleic acid dot hybridizations. Nonspecific transmission was found in 26% of the CA-RPV field isolates tested.

Barley yellow dwarf is an economically important disease wherever cereals are grown. Losses in the midwestern United States average 5–10% annually (4). The disease can be caused by any of several related luteoviruses, each of which is called barley yellow dwarf virus (BYDV) (22). The different BYDV types have been separated by specificity of transmission by various species of cereal aphids (11,17), serological relatedness (1,21), cytopathological effects in virus-infected tissue (8) and, most recently, nucleic acid size and sequence similarity (3,6,28).

Rochow (17,18) originally named the different BYDV types based on their aphid transmission specificity. He found that four types were specifically aphid-transmitted: RPV by *Rhopalosiphum padi* (L.), MAV by *Sitobion avenae* (F.), SGV by *Schizaphis graminum* (Rond.), and RMV by *R. maidis* (Fitch) (11,17). The fifth and most common BYDV type is PAV, which is transmitted nonspecifically by *Sitobion avenae, R. padi*, and *Schizaphis graminum* (17). Gildow and Rochow (7) showed that New York (NY) isolates of MAV and PAV could be transmitted by *Metopolophium dirhodum* (Walk.) but reported that a California (CA) PAV isolate was not efficiently transmitted by this aphid species.

Recent studies (7,19) have shown that field isolates are similar to the characterized BYDV types, with two important exceptions. GPV, a Chinese isolate (32), and an Australian BYDV isolate described by Lister and Sward (13) resemble MAV serologically but are transmitted also by *R. padi*. These anomalies differ from the other well-characterized BYDV types in which serotype and aphid vector specificity have been correlated regardless of the detection system used. All other identified MAV types are specifically transmitted by *Sitobion avenae* and react only with MAV antisera.

BYDV types have been identified in the past primarily through aphid transmission, although lately enzyme-linked immunosorbent assay (ELISA) has gained in popularity (4). ELISA is somewhat problematic, because antisera to the different BYDV types are not widely available, and an ELISA to SGV is a complex procedure (10,23). Nucleic acid hybridization is now more frequently used for BYDV identification, and although a cDNA clone to an Australian PAV is commercially available (BRESA, Adelaide, South Australia), clones to BYDV types in the United States are not commercially available. However, a single detection system—aphid transmission, ELISA, or nucleic acid dot blot hybridization—is generally used in field studies, because each provides BYDV type information.

In the course of a survey for BYDV types in cereals and grass weeds in California, we collected a BYDV isolate, designated CA-RPV-1, that showed unusual characteristics. This paper compares the aphid transmission, serological, and nucleic acid properties of the CA-RPV-1 strain with those of the NY-type strains of BYDV.

MATERIALS AND METHODS

Virus and vector maintenance. Type NY BYDV isolates of RPV and MAV; antisera to NY-PAV, NY-MAV, and NY-RPV; and aphid clones of *R. padi* and *Sitobion avenae* were gifts of Dr. James Duffus (USDA, Salinas, CA). NY-PAV was obtained from Dr. D. Ullman (University of California, Davis). Monoclonal antibodies reacting with NY-RPV (RPV-1, RPV-2, RPV-3), NY-MAV (MAV-1), and NY-PAV (MAV-3) were gifts of Dr. H. T. Hsu (USDA, Beltsville, MD) (9). A cDNA clone to an Australian PAV (pPA8) (15,16) was obtained from Dr. Wayne Gerlach (CSIRO, Australia). Recombinant cDNA clones to NY-PAV (pPAV 56), NY-RPV (pRPV 29), and NY-MAV (pMAV14+) (3) were gifts of Dr. Richard Lister (Purdue University).

CA-RPV-1 was isolated from *Hordeum vulgare* L. emend. Bowden 'Kombar' collected in Davis, CA. CA-PAV-2 was isolated from *Avena fatua* L., and CA-PAV-4 and CA-MAV-4 were isolated from *Aegilops juvenalis* (Thell.) Eig. CA clones of *Schizaphis graminum, M. dirhodum*, and *Sitobion avenae* were collected from Imperial Valley, Davis and Watsonville, CA, respectively. Aphids were identified by Dr. John Sorenson (California Department of Food and Agriculture). A single adult of each species was allowed to produce nymphs by membrane feeding on a 20% sucrose solution overnight. The nymphs were then used to start colonies on California Red oats (*Avena sativa* L.). Colonies were maintained at 20 C with 16 hr of light per day.

For transmission experiments, nonviruliferous aphids were given a 48-hr (5 days for *Schizaphis graminum*) acquisition access

period on detached leaves in the dark at 20 C. Either one or 10 aphids per plant were then caged on California Red oats at the two- to three-leaf stage for 5 days at 20 C in constant light. Plants were sprayed with permethrin at a concentration of 0.1%, maintained in an aphid-free greenhouse, and tested by ELISA after 2 wk.

Serological analysis. Double-antibody-sandwich (DAS) ELISA was conducted as described by Lister and Rochow (12). Immulon II plates (Dynatech, Chantilly, VA) were coated at 200 μl per well with immunoglobulin (IgG) (purified by affinity chromatography using Protein A) at 2.5 μg/ml and were incubated 3.5 hr at 37 C. Oat leaves (1 g) were ground with a sap expresser (Piedmont Tool and Die, Six Mile, SC), diluted in 0.1 M sodium phosphate buffer, pH 7 (1:4, w/v), and clarified with chloroform (1:1, v/v). Samples were applied at 200 μl per well, and plates were incubated overnight at 4 C. Alkaline phosphatase-conjugated IgG (Sigma Chemical Company, St. Louis, MO) was used at 2.0 μg/ml, 200 μl per well, and was incubated 3.5 hr at 37 C. *p*-Nitrophenyl phosphate (Sigma) was added at 0.6 mg/ml, 200 μl per well, for color development. Results were assessed spectrophotometrically by reading at 405 nm with a Titertek Multiscan MC.

Indirect ELISA was done by coating plates and processing samples as described above. Ascites fluid containing monoclonal antibody was added at 1:2,500 or 1:5,000 dilution, 200 μl per well, as a secondary antibody, and alkaline phosphatase-conjugated rabbit IgG + IgM (H&L) to mouse (Jackson Immunochemicals, Avondale, PA) was added at a 1:2,000 dilution, 200 μl per well. Substrate was added to the plates, and results were assessed as before.

Nucleic acid analysis. For nucleic acid dot blots, samples were processed as for ELISA (1 g of oat leaves to 4 ml of buffer), and 25 μl was spotted onto 0.45-μm nitrocellulose (Schleicher & Schuell, Inc., Keene, NH) (presoaked in 20× SSC, 0.3 M trisodium citrate, 3.0 M sodium chloride) with a Biodot apparatus (Bio-Rad Laboratories, Richmond, CA). Blots were baked for 1–2 hr at 80 C. Plasmids were purified according to Froman as described in Rodriguez and Tait (24) and were further purified by centrifugation in cesium chloride-ethidium bromide gradients (14). Nick translations were done with a nick translation kit and 50 μCi α-^{32}P dCTP (Amersham Corp., Arlington Heights, IL). Prehybridizations, hybridizations, washes, and exposure to film were performed following the Zeta Probe instruction manual (Bulletin 1234, Bio-Rad).

Double-stranded RNAs (dsRNAs) were isolated as in Valverde et al (26). Twenty-eight grams of BYDV-infected oats (whole plants) were ground, extracted with STE-buffered phenol (STE = 0.1 M NaCl, 0.05 M Tris, 0.001 M ethylenediaminetetraacetate), eluted through three cycles of CF-11 chromatography, concentrated by ethanol precipitation, and electrophoresed for 3 hr at 100 V in a 6% polyacrylamide gel (40:1 acrylamide:bis-acrylamide) with a vertical Mini-slab gel apparatus (Idea Scientific, Corvallis, OR). DsRNAs were visualized by staining with ethidium bromide and photographed.

To confirm the double-stranded nature of these RNAs, nuclease digestions were done on samples extracted as above by incubating them at 37 C for 10 min using 1 μg/ml RNase A (Sigma) in water (low-salt) or 2× SSC (high-salt) or using 10 μg/ml DNase I (Sigma) in 0.1 M Tris buffer, pH 7.3, containing 0.01 M MgCl. The reactions were stopped by extracting with phenol:chloroform (2:1). DsRNAs were ethanol-precipitated, electrophoresed, and visualized as before.

RESULTS

Aphid transmission characteristics. In tests using 10 aphids per plant, CA-RPV-1 was transmitted efficiently by *R. padi* from NY, *Sitobion avenae* from NY and from CA, and *Schizaphis graminum* from CA; it was not transmitted by *M. dirhodum* from CA (Fig. 1). In control experiments, NY-RPV was not transmitted by NY or CA clones of *Sitobion avenae*. In all experiments using 10 aphids per plant, CA-RPV-1 was transmitted as efficiently as NY-PAV by NY and CA clones of *Sitobion avenae*. BYDV was not transmitted by nonviruliferous aphids used as controls.

In the transmission tests using single aphids, CA-RPV-1 was also efficiently transmitted by *R. padi* and *Sitobion avenae* (Fig. 2). The percentages of transmission of CA-RPV-1 and NY-RPV by *Sitobion avenae* are significantly different at the 5% level by the chi-square test (Fig. 2). Transmission of CA-RPV-1 and NY-PAV by single aphids of *Sitobion avenae* was much less efficient than transmission by 10 aphids per plant. NY-MAV was much more efficiently transmitted by single aphids of *Sitobion avenae* than the other BYDV types (Fig. 2).

CA isolates (collected from barley, oats, wheat, and grass weeds) that reacted serologically as RPV were tested for their vector specificity in transmission tests with *Sitobion avenae* and *R. padi* from NY. Of the 34 isolates tested, nine were nonspecifically transmitted by both aphid species; the other 25 were transmitted only by *R. padi*.

Serological analysis. CA-RPV-1 reacted strongly in DAS-ELISA with antiserum to NY-RPV (Table 1). No cross-reactions were obtained with antiserum to NY-PAV or NY-MAV, and control NY-BYDV antigens reacted strongly only with their respective antisera.

The serological relatedness of CA-RPV-1 to the NY-BYDV types was further evaluated by indirect ELISA tests using monoclonal antibodies to NY-RPV, NY-PAV, and NY-MAV. CA-RPV-1 reacted strongly with monoclonal antibodies to NY-RPV, regardless of whether the virus was transmitted by *R. padi* or *Sitobion avenae* (Table 2). Interestingly, MAV polyclonal antibodies were able to trap both CA-RPV-1 and NY-RPV antigen to ELISA plates, where they reacted with monoclonal antibodies to RPV. However, no reactions were obtained in any combination using MAV or PAV monoclonal antibodies. NY-MAV reacted only to the MAV-1 monoclonal antibody and not to the MAV-2 (data not shown) or MAV-3 monoclonal antibodies, which were originally made to MAV. NY-PAV, however, reacted with MAV-2 and MAV-3 monoclonal antibodies.

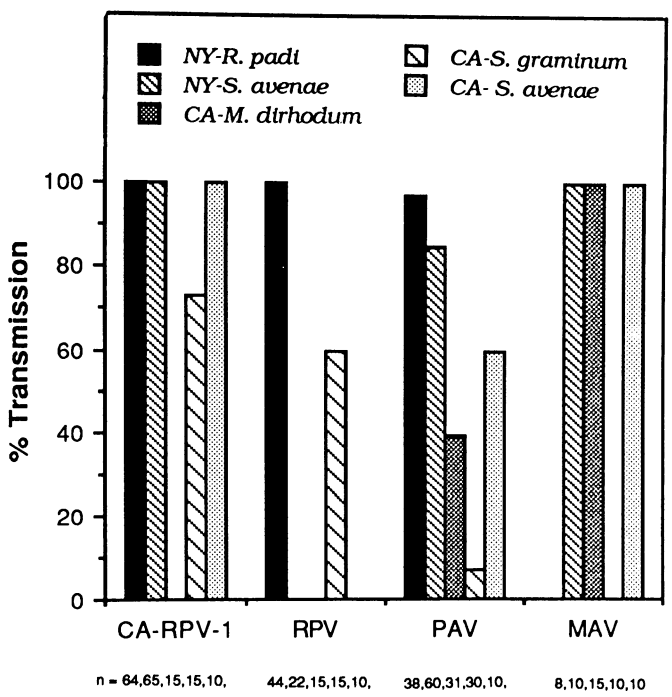

Fig. 1. Transmission of CA-RPV-1, NY-RPV, NY-PAV, and NY-MAV barley yellow dwarf virus types by New York (NY) and California (CA) aphid species using 10 aphids per plant. The data summarize experiments replicated over time; *n* = total number of plants tested for each treatment.

Nucleic acid analysis. Analysis and comparison of dsRNA profiles of CA-RPV-1 and NY-BYDV types also showed that CA-RPV-1 is essentially indistinguishable from NY-RPV (Fig. 3). The dsRNA pattern for CA-RPV-1 showed two major dsRNAs with electrophoretic mobilities identical to those of NY-RPV. In contrast, NY-PAV and NY-MAV showed a different dsRNA pattern with electrophoretic mobilities distinct from those of CA and NY RPVs. All RNAs were resistant to RNase A in 0.3 M NaCl and susceptible to RNase A in water, thus confirming their status as dsRNAs (data not shown).

Nucleic acid dot hybridization analysis showed that CA-RPV-1 does not hybridize with recombinant cDNA plasmids to NY-PAV (pPAV56), NY-MAV (pMAV14+), NY-RPV (pRPV 29) (Fig. 4), Australian PAV (pPA8) (data not shown), or beet western yellows virus (pBW1066 or pBW1085) (data not shown). Other CA isolates of RPV reacted with the NY-RPV clone in dot hybridizations but not with the clones to MAV or PAV (data not shown).

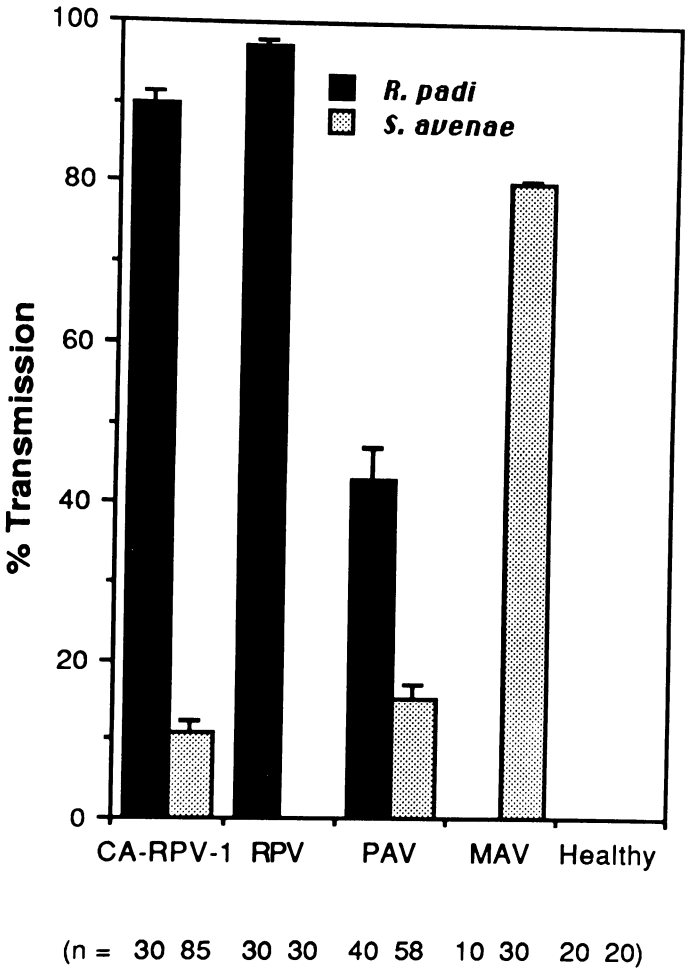

Fig. 2. Transmission of CA-RPV-1, NY-RPV, NY-PAV, and NY-MAV barley yellow dwarf virus types by single aphids of *Sitobion avenae* and *Rhopalosiphum padi* from New York. Bars represent the standard error of the mean; n = total number of plants tested for each treatment. The percentages of transmission of CA-RPV-1 and NY-RPV by *Sitobion avenae* are significantly different by the chi-square test at the 5% level.

DISCUSSION

Several reports of variation within BYDV types have been published (5,13,19,32). These reports and the work presented here reemphasize that BYDV is a group of viruses with gradations in symptoms evoked (4,7,29), transmission characteristics (2,13,30), and serological properties (13,20). Our findings and those of Lister and Sward (13) that monoclonal antibodies made to NY-MAV can no longer react with that BYDV show even more definitively that the BYDVs are a diverse group whose members do not exactly fit Rochow's (17) original definition of the RPV, PAV, and MAV types. Although our CA aphid clones transmitted similarly to their NY counterparts, this is not universally the case (7), and thus the aphid vectors are another source of variation within this viral system.

The discrepancy we found between transmission rates by 10 aphids and by single aphids has also been noted by others (25,31). We believe that the values for multiple-aphid transmission reflect field conditions more closely than those for single-aphid transmission.

TABLE 1. Serological relationships of CA-RPV-1 and New York (NY) barley yellow dwarf virus (BYDV) types in double-antibody-sandwich enzyme-linked immunosorbent assay[a]

BYDV antigen	Polyclonal rabbit antiserum[b]		
	NY-RPV	NY-PAV	NY-MAV
CA-RPV-1	1.72	0.00	0.01
NY-RPV	1.51	0.00	0.02
NY-PAV	0.27	1.00	0.20
NY-MAV	0.04	0.02	1.23
Healthy	0.02	0.00	0.00

[a] Mean absorbance at 405 nm of four wells from two separate tests, with a possible optical density range of 0.0–2.0, from a 1:4 dilution of virus-infected or uninoculated California Red oat tissue.
[b] Purified immunoglobulin (IgG) was used at 2.5 μg/ml for coating, and alkaline phosphatase-conjugated IgG was used at 2.0 μg/ml.

TABLE 2. Serological relationships of CA-RPV-1 and New York (NY) barley yellow dwarf virus (BYDV) types in indirect enzyme-linked immunosorbent assay using monoclonal and polyclonal antibodies[a]

	Primary[b] and secondary[c] antibodies											
	RPV[b]	PAV	MAV	RPV	PAV	MAV	RPV	PAV	MAV	RPV	PAV	MAV
Antigen source	RPV-1[c]			RPV-2			MAV-1			MAV-3		
CA-RPV-1 (*Rhopalosiphum padi*)[d]	1.47	0.19	0.92	0.55	0.21	0.46	0.02	0.02	0.03	0.06	0.03	0.03
CA-RPV-1 (*Sitobion avenae*)[d]	1.39	0.19	0.81	0.67	0.22	0.42	NT	NT	NT	NT	NT	NT
NY-RPV	1.40	0.15	0.94	0.87	0.23	0.51	0.26	0.03	0.04	0.01	0.00	0.00
NY-PAV	0.06	0.03	0.05	0.08	0.05	0.07	0.05	0.06	0.08	0.18	0.80	0.51
NY-MAV	0.02	0.02	0.03	0.01	0.02	0.04	0.30	1.16	1.85	0.00	0.00	0.00
Healthy oats	0.02	0.02	0.03	0.04	0.03	0.05	0.05	0.06	0.02	0.00	0.00	0.00

[a] Mean absorbance at 405 nm of two wells with a possible range of 0.00–2.00. NT = not tested.
[b] Purified rabbit polyclonal immunoglobulin to the NY-BYDV type shown was used to coat microtiter plates at 2.5 μg/ml.
[c] Monoclonal antibody ascites fluid was used as a secondary antibody at 1:5,000 (RPV-1) or 1:2,500 (MAV-1, MAV-3, and RPV-2).
[d] Designates aphid used to transmit the virus source.

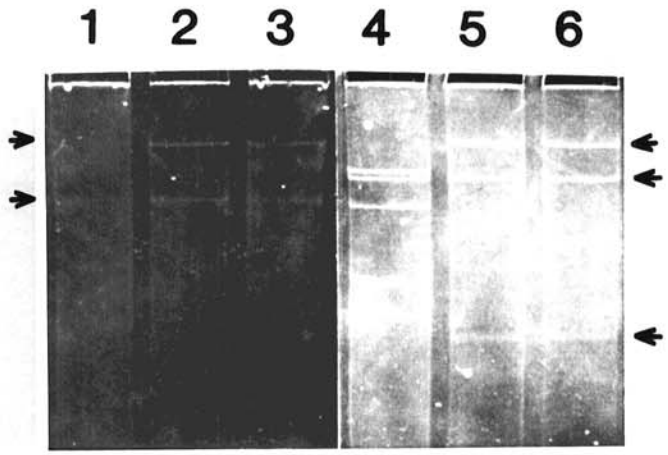

Fig. 3. Polyacrylamide gel electrophoresis of double-stranded RNAs (dsRNAs) extracted from healthy California Red oats (lane 1) and California Red oats infected with CA-RPV-1 (lane 2), NY-RPV (lane 3), CA-PAV-2 (lane 5), and NY-PAV (lane 6) isolates of barley yellow dwarf virus. Lane 4 shows dsRNAs extracted from *Nicotiana edwardsonii* infected with cucumber mosaic virus. Electrophoresis was for 3 hr at 100 V in a 6% gel. DsRNAs were detected by staining with ethidium bromide and ultraviolet illumination. Arrows at left show locations of RPV dsRNAs (molecular weights 3.6 and 1.6×10^6), and arrows at right show PAV dsRNAs (molecular weights 3.6, 2.0, and 0.5×10^6). Molecular weights are from Gildow et al (6).

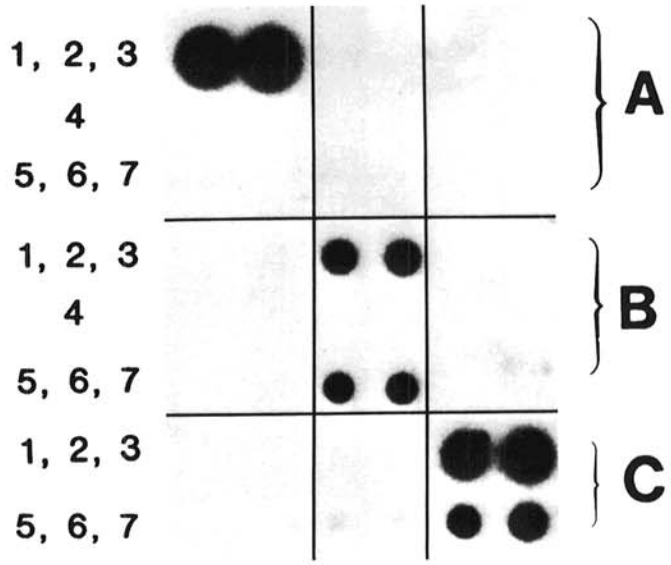

Fig. 4. Nucleic acid dot blots of New York (NY) and California (CA) barley yellow dwarf viruses (BYDVs). Samples of clarified leaf sap from infected and healthy California Red oats were spotted (25 μl) in duplicate onto 0.45-μm nitrocellulose. Each block of nitrocellulose had two dots of NY and CA isolates of a given BYDV type: 1 is NY-RPV, 2 is NY-PAV, 3 is NY-MAV, 4 is healthy oats, 5 is CA-RPV-1, 6 is CA-PAV-4, and 7 is CA-MAV-4. Hybridization was with ^{32}P-labeled, nick-translated pRPV 29 **(A)**, nick-translated pPAV 56 **(B)**, and nick-translated pMAV14+ **(C)**.

Defining a type of BYDV on the basis of its serological, hybridization, or transmission characteristics alone may not accurately reflect the true nature of the virus. A more complete analysis of any given isolate is necessary to better define the type. Valverde et al (27) used serology, dsRNA pattern, and molecular hybridization to compare luteoviruses and found that a combination of detection methods gave the greatest differentiation among them.

Although CA-RPV-1 showed no major differences from NY-RPV in serological composition or dsRNA profile, the cDNA clone to NY-RPV did not react with it. Furthermore, CA-RPV-1 was transmitted in a nonspecific manner similar to NY-PAV. Accordingly, we suggest that the classification of BYDV types be based on several characters.

The nonspecific aphid transmission described here is not rare, and other such isolates will undoubtedly be found upon closer investigation of field isolates. The epidemiological advantage of nonspecific transmission could be great under certain conditions, allowing the virus a wider host or vector range and consequently a better chance for survival.

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