Neutralization of Beet Western Yellows Virus by Antisera Against Barley Yellow Dwarf Virus

James E. Duffus and W. F. Rochow

Plant Pathologist, Western Region, Agricultural Research Service, U.S. Department of Agriculture, U.S. Agricultural Research Station, P. O. Box 5098, Salinas, CA 93901; and Research Plant Pathologist, Northeastern Region, Agricultural Research Service, Department of Agriculture, and Professor of Plant Pathology, Cornell University, Ithaca, NY 14853.

Cooperative Investigation, Agricultural Research Service, U.S. Department of Agriculture, and Cornell University Agricultural Experiment Station. Supported in part by NSF Grant PCM74-19814.

Mention of a trademark, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U. S. Department of Agriculture, and does not imply approval of it to the exclusion of other products that also may be suitable.

Accepted for publication 21 June 1977.

ABSTRACT

DUFFUS, J. E., and W. F. ROCHOW. 1978. Neutralization of beet western yellows virus by antisera against barley yellow dwarf virus. Phytopathology 68:45-49.

A possible relationship between beet western yellows virus (BWYV), a damaging virus of dicotyledonous plants, and barley yellow dwarf virus (BYDV), a virus of major economic importance in monocotyledonous grains and grasses was suspected on the basis of their biological properties. Antisera prepared against the MAV, RPV, and PAV isolates of BYDV in New York were tested against four isolates (ST-1, ST-7, ST-9, and E-4) of BWYV in California. Virus-antiserum mixtures were subjected to density-gradient centrifugation, analyzed photometrically, and tested for virus neutralization. Antisera prepared against the three isolates of BYDV reduced or eliminated virus antigen in scanning patterns and reduced or eliminated virus in the normal BWYV-bearing zones. Antiserum against healthy shepherd's purse or oat juice did not affect scanning patterns or infectivity. In contrast to the reaction of BYDV antiserum, none of 29 different antisera to 23 different viruses neutralized BWYV. In five of 14 tests *Myzus persicae* transmitted BWYV to oats (*Avena byzantina*). In nine of 20 tests *Macrosiphum avenae* transmitted BWYV to shepherd's purse. Inoculations of over 850 BWYV host plants failed to establish that the PAV, RPV, or MAV isolates of BYDV could infect them.

The yellowing virus diseases are emerging as the most important group of virus diseases in plants. Despite the difficulties involved in assessing damage and etiology, serious yellows diseases are known for barley, oats, grapes, potatoes, spinach, and sugarbeets (21). Beet western yellows virus (BWYV) induces stunting and chlorosis of a wide range of dicotyledonous species and is probably the most economically important virus on this group of plants (8). Barley yellow dwarf virus (BYDV) is a virus of major economic importance in the monocotyledonous grains and grasses.

The two viruses, BWYV and BYDV, are similar in symptoms, vector transmission characteristics, particle morphology, and virus localization within phloem tissues, but differ markedly in plant host range and vector species. We recognized in the late 1960's that serological comparisons of these two virus groups would be valuable because criteria for relationship other than biological behavior were lacking.

Infectivity neutralization by antiviral antibodies had been used successfully in serological comparisons among BWYV isolates in California (7, 9, 10, 11, 13, 14, 15, 16) and among BYDV isolates in New York (20). An exchange of antiseria, virus isolates, and aphid vectors was arranged early in 1971. We report here the results of serological and aphid-transmission tests with BYDV and BWYV conducted in California since then. A preliminary report of some of these studies has been published (7, 12).

0032-949X/78/000007 $03.00/0

Copyright © 1978 The American Phytopathological Society, 3340 Pilot Knob Road, St. Paul, MN 55121. All rights reserved.

MATERIALS AND METHODS

The BWYV isolates tested in these studies were: ST-1 and ST-7 (originally from radish, *Raphanus sativus* L.); isolate ST-9 (originally from broccoli, *Brassica oleracea* var. *botrytis* L.); and isolate E-4 (originally from turnip, *Brassica rapa* L.). These virus isolates were maintained in desiccated plant tissue.

The BYDV isolates (MAV, PAV, and RPV) were maintained in oats (*Avena byzantina* C. Koch) (17).

For activation of BWYV isolates, desiccated tissue was ground in 0.05 M phosphate buffer (pH 7.0) containing 0.01 M glycine in the proportion of one part plant tissue (fresh weight basis) to one part diluent. These extracts were placed directly on sucrose density gradients (20-60%), centrifuged 2 hr at 73,450 *g* in a SW 50.1 rotor, and, after dilution with buffer to 20% sucrose, the virus zones (18-26 mm from the top of the tubes) were fed to aphids (6).

Nonviruliferous green peach aphids [*Myzus persicae* (Sulz.)] were reared on radish and *Rhopalosiphum padi*

(L.) and *Macrosiphum avenae* (Fab.) were maintained on virus-free oats.

The handling of aphids, isolates of BWYV, membrane feeding techniques, and antigen and antiserum preparation were as previously reported (1, 9, 16). Extracts of BWYV for antigen preparations, virus neutralization, and antigen scanning-pattern analysis were prepared from infected shepherd's purse, *Capsella bursa-pastoris* (L.) Medic. Fresh plant material was ground in a food grinder 1:1 (w/v) with 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine, and then homogenized at 45,000 rpm in a VirTis homogenizer. The resulting crude extracts were heated to 45 C and clarified by low-speed centrifugation (20 min at 12,100 *g*). Clarified juice was ultracentrifuged (2 hr at 80,800 *g*). Pellets were resuspended in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine.

Gradient columns for scanning-pattern analysis were made by layering 4, 7, 7, and 7 ml, respectively, of 10, 20, 30, and 40% sucrose in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine. Centrifugation was done in a SW-25 rotor for 4 hr at 58,600 *g*. The gradient columns were scanned photometrically with an ISCO Model D density-gradient fractionator using the sensitive scale (A_{254} = 0.05). Density-gradient centrifugation for virus neutralization tests was done in a SW 50.1 rotor for 2 hr at 73,450 *g*. Gradient columns were prepared by layering 0.9 ml each of 20, 30, 40, 50, and 60% sucrose in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine. Virus samples were mixed with equal volumes of serum (diluted 1:5) and incubated 0.5 hr at 37 C. Samples for neutralization tests were removed from the zone 18-26 mm from the top of the tubes by means of a j-shaped hypodermic needle.

All density-gradient fractions used for membrane feeding were adjusted to 20% sucrose (by dilution with buffer) before they were placed on the membranes. Preparations thus diluted were concentrated about 14 times greater than the crude juice.

Aphids that had fed through membranes were tested for virus transmissibility to shepherd's purse. Control aphids (from the same colony) which did not have access to the virus also were tested on that host as controls.

Antisera to isolates of BWYV were prepared in Salinas (10, 16); antisera to BYDV were prepared in Ithaca (18). Unless otherwise specified, antisera were diluted 1:5 in saline. Other sera used in these studies were obtained from A. H. Gold (Dept. of Plant Pathology, University of California, Berkeley), or were from various sources in different parts of the world. Serological reactions were evaluated in two ways. Virus neutralization was assayed biologically by tests for virus activity following serological reactions (16, 19). In a physical assay, the amount of unreacted virus was estimated photometrically (2, 7).

Host range was studied by inoculation of a number of individual BWYV indicator plants by means of 20-30 viruliferous aphids reared on oat plants infected with either the MAV, the PAV, or the RPV isolate of BYDV. The aphids were permitted to feed on the test plants for 48 hr, and then were killed with a nicotine sulfate spray. Oats (cultivar Coast Black) were inoculated by means of aphids in each series of tests as verification that the aphids carried the different isolates of BYDV. Recovery of the virus isolates from each inoculated plant was attempted 5-6 wk after inoculation. In transmission and recovery attempts, *M. avenae* was used with the MAV isolate, whereas *R. padi* was used with the RPV and PAV isolates.

RESULTS

Serological tests.—In the first series of tests, antisera prepared against the MAV, RPV, and PAV isolates of BYDV were tested against three isolates of BWYV. Antisera prepared against the same isolates of BWYV, healthy oats, and healthy shepherd's purse were tested against the BWYV isolates and served as controls (Table 1). The virus-antiserum mixtures were subjected to density-gradient centrifugation and tested for virus neutralization (16).

TABLE 1. Serological interactions of barley yellow dwarf virus (BYDV) antiserum with beet western yellows virus (BWYV)

Virus isolate	Relative virus dilution	Infectivity of virus zone after incubation with the indicated serum[a]								
		MAV	RPV	PAV	HO	ST-7	ST-1	E-4	HSP	Aphid[d] control
ST-7[b]	1:1	2[c]	0	2	24	2	3	1	24	0
	1:2	3	0	9	22	3	3	0	22	0
ST-1	1:1	0	0	4	24	0	0	0	24	0
	1:2	4	0	2	22	0	0	1	18	0
E-4	1:1	0	0	7	24	10	7	1	24	0
	1:2	4	2	5	24	2	6	0	23	0

[a]Antisera against different isolates of BYDV (MAV, RPV, and PAV) and BWYV (ST-7, ST-1, and E-4) and controls (HO = healthy oats, HSP = healthy shepherd's purse).

[b]The virus samples were obtained from infected shepherd's purse, clarified by low-speed centrifugation and pelleted by ultracentrifugation. Pellets were resuspended in buffer to about 6.0% of the original volume of sap. Portions of the resulting virus samples were diluted with equal volumes of serum (diluted 1:5) and incubated 0.5 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation (2 hr at 73,450 *g*), and samples for infectivity assays were removed from the zone 18-26 mm from the tops of SW-50.1 tubes (13 × 51 mm).

[c]The number of shepherd's purse plants infected of 24 inoculated with treated virus, in two tests, by means of groups of 25 green peach aphids fed through membranes on each sample.

[d]Aphids directly from healthy colony were placed on shepherd's purse indicator plants.

Antisera prepared against the three isolates of BYDV effectively neutralized the three BWYV isolates (ST-1, ST-7, and E-4) in a manner identical to the homologous BWYV antiserum. Antiserum against healthy oats and healthy shepherd's purse did not affect infectivity.

In a second series of tests, virus-antiserum mixtures were subjected to density-gradient centrifugation, analyzed photometrically (2, 11), and tested for virus neutralization. Virus antigen was reduced or eliminated in the scanning patterns of the sucrose density-gradient columns and infectivity in the normal virus-bearing zones was reduced or eliminated when antiserum against any of the three BYDV isolates was reacted with any of the three BWYV isolates.

In our earlier tests in New York (20), antiserum against the E-4 isolate of BWYV reacted with the RPV isolate of BYDV, but antisera against ST-1 and ST-7 did not. It seemed probable that the two nonreacting BWYV antisera also had lower homologous titers than the E-4 antiserum. This hypothesis was confirmed by a series of antiserum dilution tests in which the original antiserum against BWYV isolate ST-1 that had been sent to New York and two antisera produced later against higher concentrations of the same isolate were tested with four BWYV isolates (Table 2).

Also, in the earlier tests, antiserum against the RPV isolate of BYDV was more effective than antiserum against either the MAV or PAV isolates in neutralizing the infectivity of the three isolates of BWYV. Similar results were obtained with different BWYV antisera in the present series of tests (Table 1).

In a series of antiserum dilution tests in which antisera against the MAV, PAV, and RPV isolates of BYDV were tested with the four BWYV isolates (Table 3), reactions were identical, as in the earlier tests with BWYV antisera of different titers. However, although the greater effectiveness of RPV antiserum (compared with MAV and PAV antisera) in neutralizing the infectivity of BWYV isolates appears to depend on titer, the RPV and MAV antisera apparently do not differ in titers for homologous antigen (1). On the basis of these tests and those of the reciprocal reaction in New York (20), it appears that BWYV is more closely related to the RPV isolate than to either the MAV or PAV isolates

For a number of years virus neutralization has been used as a highly specific test for persistent viruses of beet and for BYDV. It also has been used as a specific indicator for separation of individual viruses from groups of animal viruses (3). However, the reaction between BYDV antisera and BWYV, and the reciprocal reaction between BWYV antisera and the RPV strain of BYDV (20), prompted an investigation of the reactions with BWYV of antisera against apparently unrelated viruses. Antisera from a wide range of sources from North America and Europe, produced and preserved in various ways, were tested with the E-4 isolate of BWYV. Antisera against the E-4 isolate of BWYV; the MAV, RPV, and PAV isolates of BYDV; healthy oats; and healthy shepherd's purse served as controls.

Antisera against the E-4 isolate of BWYV and the MAV, RPV, and PAV isolates of BYDV neutralized all or part of the infectivity of BWYV. All other antisera tested [including antisera against the viruses that cause alfalfa mosaic, arabis mosaic, beet curly top, beet yellows, beet yellow stunt, carnation mottle, cauliflower mosaic, cherry leaf roll, citrus psorosis, clover (white) mosaic, clover (red) vein mosaic, cucumber (wild) mosaic, pea enation mosaic, potato leaf roll, potato virus S, potato virus X, potato virus Y, squash mosaic, tobacco nucrosis, tobacco rattle, turnip mosaic, turnip yellow mosaic, and

TABLE 2. Reactions at various dilutions of three antisera of different titers produced against the ST-1 isolate of beet western yellows virus with four isolates of beet western yellows virus

Antiserum[a]	Virus isolate[b]	Infectivity of virus zone after incubation with serum at indicated dilution or with saline					
		1:5	1:25	1:125	1:625	1:3125	Saline
ST-1-1	ST-1	0[c]	10	12	12	12	12
	ST-7	0	8	11	12	12	12
	E-4	1	12	12	12	12	11
	ST-9	11	11	12	12	12	12
ST-1-2	ST-1	1	0	0	5	12	12
	ST-7	0	0	0	5	12	12
	E-4	0	0	2	9	12	12
	ST-9	0	0	0	9	12	11
ST-1-3	ST-1	0	0	0	0	7	8
	ST-7	0	0	0	0	11	10
	E-4	1	0	0	11	12	12
	ST-9	1	1	0	7	10	11

[a]Three antisera produced at different times against the same isolate of beet western yellows virus. Antiserum ST-1-1 is a low-titer serum used in earlier tests in New York and California.

[b]The virus samples were obtained from infected shepherd's purse, clarified by low-speed centrifugation and pelleted by ultracentrifugation. Pellets were resuspended in buffer to approximately 3.0% the original volume of sap. The resulting virus samples were mixed with equal volumes of serum and incubated 0.5 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation (2 hr at 73,450 *g*), and samples for infectivity assays were removed from the zone 18-26 mm from the top of SW-50.1 tubes (13 × 51 mm).

[c]The number of shepherd's purse plants infected of 12 inoculated with treated virus by means of groups of 25 green peach aphids acquisition fed through membranes on each sample.

watermelon mosaic, and antisera against healthy oats and healthy shepherd's purse] did not reduce BWYV infectivity.

Aphid transmission tests.—Early studies (5) had demonstrated transmission of BWYV by two species of grain aphids, *Acyrthosiphon dirhodum* and *Macrosiphum avenae*. The demonstrated serological relationship between BWYV and isolates of BYDV prompted reinvestigation of some aspects of transmission of these two viruses in New York (20) and in California, where *M. avenae*, *Myzus persicae*, and *R. padi* were used in attempts to transmit four isolates of BWYV, with various combinations of aphids, source plants, and test plants. In five of 14 tests, *M. persicae* transmitted two isolates of BWYV (E-4 and ST-1) to Coast Black oats. Infected oats were symptomless, but virus was recovered from 23 of the 672 oat plants tested. *Macrosiphum avenae* or *R. padi* in parallel tests failed to transmit any of the BWYV isolates to oats.

In 9 of 20 tests, *M. avenae* transmitted all four BWYV isolates from shepherd's purse source plants to shepherd's purse test plants. A total of 22 of 320 inoculated test plants showed BWYV symptoms. In parallel tests, *R. padi* failed to transmit BWYV from shepherd's purse to shepherd's purse.

Host-range tests of BYDV in the Dicotyledoneae.—Although BYDV has a wide host range in the Gramineae, apparently little effort has been made to investigate its host range in the Dicotyledoneae. The demonstration of serological relationships between BWYV and BYDV prompted an investigation of the possible host range of BYDV in certain key indicator hosts of BWYV.

Over 850 plants of the following species were inoculated with the MAV, RPV, and PAV isolates of BYDV: BORAGINACEAE: *Amsinckia douglasiana* DC.; CHENOPODIACEAE: *Beta vulgaris* L., *Chenopodium quinoa* L., *Spinacia oleracea* L.; COMPOSITAE: *Lactuca sativa* L.; CRUCIFERAE: *Brassica juncea* (L.) Czern., *B. kaber* (DC.) L. D. Wheeler, *B. rapa* L., *Capsella bursa-pastoris* (L.) Medic., *Crambe abyssinica* Hochst. ex R. E. Fries, *Lepidium nitidum* Nutt., *Raphanus sativus* L., *Thlaspi arvense* L.; SOLANACEAE: *Datura stramonium* L.

The BYDV was never recovered from any inoculated plant.

DISCUSSION

Previous serological studies have demonstrated a close reciprocal relationship between BWYV as it occurs in the United States and Europe and malva yellows virus (Duffus, *unpublished*), turnip yellows virus (TuYV) from England and Germany (14), and beet mild yellowing virus (BMYV) from England (15) and Germany (Duffus and Nagi, *unpublished*). A reciprocal relationship between BWYV and the RPV isolate of BYDV has been established in the present studies and in parallel ones (20). Antiserum against the MAV and PAV isolates of BYDV reacted positively with BWYV but the reciprocal reaction was negative (20). Recent studies showed that the soybean dwarf virus from Japan was serologically related to BWYV from the United States and Europe, BMYV from Europe, TuYV from Europe, and the RPV isolate of BYDV (7). Previous serological comparisons of the RPV, MAV, and PAV isolates of BYDV showed that RPV was distinct from the other two, which were related but not identical (1, 18). Thus, the RPV isolate of BYDV apparently is more closely related to BWYV than to the PAV and MAV isolates of BYDV.

The RPV, MAV, and PAV isolates are interrelated serologically through their common reactions with BWYV, but the interrelationships are obscure in direct

TABLE 3. Reactions of various dilutions of antisera against three isolates of barley yellow dwarf virus with beet western yellows virus

Antiserum[a]	Virus isolate[b]	Infectivity of virus zone after incubation with serum at indicated dilution or with saline					
		1:5	1:25	1:125	1:625	1:3125	Saline
MAV	ST-1	0[c]	10	12	11	12	11
	ST-7	1	10	12	12	12	12
	E-4	0	9	12	12	12	12
	ST-9	7	12	12	12	12	12
PAV	ST-1	2	11	12	12	12	12
	ST-7	1	12	12	11	12	11
	E-4	3	11	12	11	12	12
	ST-9	11	12	12	12	12	12
RPV	ST-1	0	0	0	1	12	12
	ST-7	0	0	0	1	12	12
	E-4	0	1	0	1	12	12
	ST-9	0	1	4	12	12	12

[a]Antisera against MAV, PAV, and RPV isolates of barley yellow dwarf virus.

[b]The virus samples were obtained from infected shepherd's purse, clarified by low-speed centrifugation and pelleted by ultracentrifugation. Pellets were resuspended in buffer to approximately 3.0% the original volume of sap. The resulting virus samples were mixed with equal volumes of serum and incubated 0.5 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation (2 hr at 73,450 *g*), and samples for infectivity assays were removed from the zone 18-26 mm from the top of SW-50.1 tubes (13 × 51 mm).

[c]The number of shepherd's purse plants infected of 12 inoculated with treated virus by means of groups of 25 green peach aphids acquisition-fed through membranes on each sample.

serological comparisons of the BYDV isolates. These findings suggest that BWYV is intermediate between the two groups of BYDV isolates.

Neutralization of infectivity by immune serum has been used with the animal viruses longer than any other serological test, and its result is usually taken as the final, decisivive criterion for the serotype of a viral isolate. The test is considered to be the most universally specific of the immunological techniques in use (3). Chester (4) reported neutralization of infectivity in plant viruses, but most subsequent studies of antisera to plant viruses as diagnostic tools have used reactions other than virus neutralization. Nevertheless, the specificity of the neutralization reaction, as demonstrated here and in previous studies of the aphid-transmitted yellowing viruses, indicates that the method has important potential for diagnosis, especially with viruses that are difficult to isolate in quantity.

LITERATURE CITED

1. AAPOLA, A. I. E., and W. F. ROCHOW. 1971. Relationships among three isolates of barley yellow dwarf virus. Virology 46:127-141.
2. BALL, E. M., and M. K. BRAKKE. 1969. Analysis of antigen-antibody reactions of two plant viruses by density-gradient centrifugation and electron microscopy. Virology 39:746-758.
3. CASALS, J. 1967. Immunological techniques for animal viruses. Pages 113-198 *in* K. Maramorosch and H. Koprowski, eds. Methods in virology, Vol. III. Academic Press, New York. 677 p.
4. CHESTER, K. S. 1934. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic by imune sera. Phytopathology 24:1180-1202.
5. DUFFUS, J. E. 1960. Radish yellows, a disease of radish, sugar beet, and other crops. Phytopathology 50:389-394.
6. DUFFUS, J. E. 1969. Membrane feeding used in determining the properties of beet western yellows virus. Phytopathology 59:1668-1669.
7. DUFFUS, J. E. 1977. Serological relationships among beet western yellows, barley yellow dwarf, and soybean dwarf viruses. Phytopathology 67:1197-1201.
8. DUFFUS, J. E. 1977. Aphids, viruses, and the yellow plague. Pages 361-383 *in* K. F. Harris and K. Maramorosch, eds. Aphids as virus vectors. Academic Press, New York. 559 p.
9. DUFFUS, J. E., and A. H. GOLD. 1965. Transmission of beet western yellows virus by aphids feeding through a membrane. Virology 27:388-390.
10. DUFFUS, J. E., and A. H. GOLD. 1969. Membrane feeding and infectivity neutralization used in a serological comparison of potato leaf roll and beet western yellows viruses. Virology 37:150-153.
11. DUFFUS, J. E., and G. M. MILBRATH. 1977. Susceptibility and immunity in soybean to beet western yellows virus. Phytopathology 67:269-272.
12. DUFFUS, J. E., and W. F. ROCHOW. 1973. Positive infectivity neutralization reactions between isolates of beet western yellows virus and antisera against barley yellows dwarf virus. Abstract No. 0895 *in* Abstracts of Papers, 2nd Int. Congr. Plant Pathol., 5-12 Sept., Minneapolis, Minnesota (unpaged).
13. DUFFUS, J. E., and G. E. RUSSELL. 1970. Serological and host range evidence for the occurrence of beet western yellows virus in Europe. Phytopathology 60:1199-1202.
14. DUFFUS, J. E., and G. E. RUSSELL. 1972. Serological relationship between beet western yellows and turnip yellows viruses. Phytopathology 62:1274-1277.
15. DUFFUS, J. E., and G. E. RUSSELL. 1975. Serological relationship between beet western yellows and beet mild yellowing viruses. Phytopathology 65:811-815.
16. GOLD, A. H., and J. E. DUFFUS. 1967. Infectivity neutralization–a serological method as applied to persistent viruses of beets. Virology 31:308-313.
17. ROCHOW, W. F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.
18. ROCHOW, W. F., A. I. E. AAPOLA, M. K. BRAKKE, and L. E. CARMICHAEL. 1971. Purification and antigenicity of three isolates of barley yellow dwarf virus. Virology 46:117-126.
19. ROCHOW, W. F., and E. M. BALL. 1967. Serological blocking of aphid transmission of barley yellow dwarf virus. Virology 33:359-362.
20. ROCHOW, W. F., and J. E. DUFFUS. 1977. Relationships between barley yellow dwarf and beet western yellows viruses. Phytopathology 67:51-58.
21. U. S. DEPARTMENT OF AGRICULTURE. 1965. Losses in agriculture. U. S. Dep. Agric. Handb. 291. 120 p.