Production and Use of Antibodies from Hen Eggs for the SGV Isolate of Barley Yellow Dwarf Virus

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Cooperative investigation, Agricultural Research Service, U.S. Department of Agriculture, Cornell University Agricultural Experiment Station. Supported in part by NSF Grant PCM 8308672, by Rockefeller Foundation Grant 82037-18, and by USDA Grant NY(C) 157424.

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We are grateful to Irmgard Muller, Laura Tufford, Dawn Smith, Guang-he Zhou, and Forrest Sanders for excellent technical assistance.
Accepted for publication 12 March 1985.

ABSTRACT

Hu, J. S., Rochow, W. F., and Dietert, R. R. 1985. Production and use of antibodies from hen eggs for the SGV isolate of barley yellow dwarf virus. Phytopathology 75:914-919.

Antibodies were separated from yolks of 216 eggs produced by a 'Babcock 300' hen immunized with less than 100 μg of the SGV isolate of barley yellow dwarf virus (BYDV). Although antibody titer was relatively low, it remained reasonably constant during the 15 mo of egg collection. Only purified immunoglobulin was useful in the coating step of direct (double antibody sandwich) enzyme immunosorbent assays (EIA). Unpurified anti-SGV immunoglobulin was useful in indirect EIA based on trapping SGV with a monoclonal antibody that reacts with both SGV and the MAV isolate. Five kinds of serological tests showed that SGV is related to MAV and PAV, but distinct from RPV and RMV, four other isolates of BYDV used for comparison. The indirect EIA provided an assay for SGV in the presence of PAV, so we were able to determine that PAV is not a helper virus for dependent transmission of SGV by the aphid *Rhopalosiphum padi* from mixed infections of SGV and PAV.

Among the five characterized isolates of barley yellow dwarf virus (BYDV) under study at Cornell, SGV has been the most difficult to analyze. Its transmission is more variable and its purification is more tedious than that of the other isolates. The SGV isolate is transmitted specifically by *Schizaphis graminum* (Rondani), but only certain aphid biotypes (16) and only young instars are efficient vectors (25). We use the same purification methods for all five isolates, but SGV yields are much lower than those of the others (20). Another feature that sets SGV apart is the relative scarcity of SGV-like isolates in nature, an observation qualified by the fact that most studies have been done in areas where the vector is rare (4,17,19).

Although we know relatively little about SGV, several lines of evidence indicate that it is related to MAV and PAV, but distinct from RPV and RMV, four other isolates that cause barley yellow dwarf (17,23). This serological relationship is based only on weak heterologous reactions in enzyme-linked immunosorbent assays (EIA) with polyclonal and monoclonal antibodies (7,22), since we previously had not produced antiserum against SGV. In recent studies researchers have described the advantages of using female chickens to make antibodies against plant viruses (2,15,24). Advantages include production of large amounts of antibody concentrated in egg yolks after injection of relatively small amounts of virus, and suitability of this antibody in indirect EIA with rabbit antibodies. Antibody derived from egg yolks, which is only of the 7S type (15), occasionally has been termed IgY (11,12), but has been more widely accepted as IgG (2,10).

Here we describe production of antibodies against SGV in hens, evaluate assays useful with the antibodies, analyze the serological relationship of SGV to each of four other BYDV isolates, and study the role of PAV as a helper virus for dependent transmission of SGV.

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MATERIALS AND METHODS

General. The four aphid vectors, the five isolates of BYDV, and methods used to maintain them have been described (1,9,22,23). The isolates and their vectors are: SGV, transmitted specifically by *Schizaphis graminum* (Rondani); MAV, transmitted specifically by *Sitobion* (=*Macrosiphum*) *avenae* (F.); RPV, transmitted specifically by *Rhopalosiphum padi* (L.); RMV, transmitted specifically by *R. maidis* (Fitch); and PAV, transmitted by *R. padi, S. avenae*, and *S. graminum*. The test plant was Coast Black oats (*Avena byzantina* Koch). Most virus transmission tests were based on a two-day acquisition feeding on detached leaves at 15 C, and a five-day inoculation test feeding on 6-day-old seedlings at 21 C. In experiments on dependent virus transmission, plants were inoculated with SGV, PAV, or a mixture of the two viruses by infesting seedlings with appropriate viruliferous aphid vectors. Half-leaves were used for acquisition to compare virus transmission by two vectors (18).

Clarified virus preparations were made by preparing 3 g of finely chopped tissue in 9 ml of 0.02 M phosphate-buffered saline, pH 7.4, containing 0.05% Tween-20, then grinding for 6 sec in the PT-20 probe of a Brinkmann Polytron Homogenizer. Next each sample was shaken by hand with 9 ml of chloroform and centrifuged at low speed to break the emulsion. Purified virus and healthy control preparations were made, as previously described, by chloroform clarification, differential centrifugation, and sucrose gradient centrifugation (20).

Production and preparation of antibodies against SGV. Purified preparations of SGV collected from zones in sucrose gradient tubes and emulsified with Freund's incomplete adjuvant were used to immunize each of two adult 6- to 8-mo-old female 'Babcock 300' chickens. Both were in production and kept in single battery cages with feed and water ad libitum. Each emulsion was injected (about 0.5 ml per site) into both sides of the breast and into one leg. Injections were repeated 14 days later as a booster. Each hen

received a total of about 30 μg of SGV in the first multiple site immunization and about 64 μg in the secondary immunization. These virus preparations, which represented over 16 kg of infected plant tissue, had been accumulated over several months as frozen concentrates from more than 14 L of clarified juice (20). Each virus preparation was assayed in injection (14) and membrane-feeding tests (20,21) with the four aphid species. These assays showed that only SGV was injected into the hens.

One hen died prior to any significant production of eggs. The other ceased production for approximately seven days, but then layed about one egg each day except during periods of molting. We collected and processed 216 eggs during 468 days. The eggs were used in groups of two to four to prepare antibody from the yolks, usually by a simple chloroform clarification method (13). Each group of yolks was rinsed with distilled water, combined with an equal volume of neutral 0.01 M phosphate buffered physiological saline, and stirred with a volume of chloroform equal to that of the mixture. Emulsions were broken by low-speed centrifugation and supernatants were kept separate for testing. Since IgG is the main immunoglobulin in the yolks (2,10), these preparations will be called crude IgG here. Separate preparations were later combined for use and for storage at −20 C. In one experiment, preparations made with chloroform were compared with parallel preparations made with use of polyethylene glycol (PEG) (MW 8,000) (15).

Some experiments were done with two pools of crude IgG made by combining individual preparations. Aliquots of these pools were stored at −20 C. One of these pools (crude IgG-A) was made by combining parts of 36 preparations from eggs collected between 10 and 193 days after the booster injection. The other (crude IgG-B) was a combination of five preparations from eggs collected 213–262 days after the booster.

Antibody titers of the 57 preparations were estimated in heterologous tests with MAV. These tests were done by mixing 0.8 ml of a clarified preparation of MAV (antigen) with 0.2 ml of each undiluted antibody preparation. This mixture was incubated at 37 C for 30 min, stored overnight at 4 C, and then used in a double antibody sandwich (direct) EIA to estimate the amount of unreacted MAV. Controls in each test included saline, preparations from yolks of normal eggs from other hens, crude IgG preparations tested in previous titrations, and anti-MAV immunoglobulin from rabbits.

Immunoglobulin was prepared from some of the crude IgG preparations as described previously (22). Before precipitation with ammonium sulfate, the preparation was incubated with a concentrate of healthy oats to remove antibodies against normal oat components. Some of this preparation, which will be called precipitated IgG, was conjugated with type VII alkaline phosphatase (22).

Precipitated IgG was also used to prepare purified IgG with a DEAE-Sephacel ion-exchange column (6). The 5-ml column was equilibrated with 10 mM tris-HCl, pH 8.0, and globulin was eluted with a linear gradient of 0 to 500 mM NaCl in the tris-HCl buffer.

Enzyme-immunosorbent assay. Direct EIA with rabbit antibodies was carried out as described previously (19,22). Immulon I polystyrene substrate plates (Dynatech Laboratories Inc.) with 100 μl of liquid per round-bottom well were used for most tests. The coating step (immunoglobulin at 10 μg/ml) was at 37 C for about 6 hr, antigens were incubated at 4 C overnight, conjugated antibodies (diluted from 1:800 to 1:3,200) were incubated at 37 C for about 5 hr, and the alkaline phosphatase reaction was measured after 45 min at room temperature with a Dynatech model MR-580 Microelisa Reader at 405 nm. Similar procedures were used in direct EIA with SGV hen antibody preparations, but many variations of incubation temperatures, times, and other factors were studied as described below.

In the indirect EIA developed for SGV, plates were first coated with a 1:1,000 dilution of mAB-MAV4, a monoclonal antibody that reacts with both MAV and SGV (7), and incubated at 37 C for 4 hr. Antigen was incubated overnight at 4 C, precipitated IgG (2.5 μg/ml) was incubated for 3.5 hr at 37 C, and alkaline phosphatase-conjugated rabbit anti-chicken immunoglobulin (diluted 1:300) (Dynatech Diagnostics, Inc.) was added for 3.5 hr at 37 C before substrate was added for 45 min at room temperature.

Controls in all EIA tests included healthy oat preparations, buffer, and purified antigen of known amounts of virus. At least two wells were used for each sample.

Other serological assays. In some experiments absorption reactions were done in test tubes. Clarified virus preparations were mixed with crude IgG, incubated at 37 C for 30 min, and kept overnight at 4 C prior to assay by two methods. In one, virus-antibody mixtures were evaluated by direct EIA to measure unreacted virus with homologous rabbit antibodies except that anti-MAV immunoglobulin was usually used for SGV. In the other, portions of each virus-antibody mixture were assayed by means of aphids (starved 6 hr) fed through stretched Parafilm (overnight at 15 C) on virus-antibody mixtures (1:2 in buffered sucrose, 20%) (20,21). Then the aphids (10 per plant) were given an inoculation test feeding of 5 days. Some comparisons were by immunodiffusion tests (1% ionagar) with concentrated virus preparations and various virus-specific antisera (1).

RESULTS

Preparation of IgG from egg yolks. We made preparations of crude IgG from 57 sequential groups of two to four eggs from 216 eggs collected during 15 mo. Since our initial attempts to use these preparations in direct EIA were unsuccessful, we had no easy way to titrate each preparation because shortage of SGV precluded use of other kinds of serological tests. We compared some preparations by measuring their capacity to absorb antigen when mixed with SGV or MAV. In these tests, residual, unreacted antigen was assayed by direct EIA with anti-MAV immunoglobulins. Results of one such experiment (Table 1) showed that the preparations contained SGV antibodies, that the antibodies also reacted with MAV, and that they blocked SGV transmission by aphids. Since results with SGV were inconsistent and EIA reactions were weak, we used MAV antigen to estimate the titer of each crude anti-SGV IgG preparation.

Because this heterologous absorption was a relatively crude way to titrate the IgG preparations, we evaluated its adequacy by comparing dilutions of crude IgG-B, by using different concentrations of MAV, and by testing a centrifugation step between the absorption reaction and the EIA assay done the next day. Results were the same whether or not samples were centrifuged at low speed; thus, we did not remove the small precipitate from each tube in subsequent tests. Results of comparisons of twofold dilutions of crude IgG made in preparations of normal yolks showed that such differences were readily detected with a range of MAV concentrations. For example, when MAV was used at 0.25 μg/ml, mean absorbance values were 11, 22, 38, and 46% of the yolk control (0.558) for

TABLE 1. Presence of anti-SGV antibodies in hen yolk preparations detected in two ways after absorption of virus antigen

Preparation tested[a]	Absorbance at 405 nm in direct EIA with MAV-globulin and virus shown[b]		Infectivity of SGV[c]
	MAV	SGV	
Crude IgG	0.032	−0.040	0
Crude control	1.064	0.138	8
Precipitated IgG	0.021	−0.045	0
Precipitated control	0.990	0.145	9
Healthy control	0.026	0.017	0

[a] Controls were preparations of eggs from nonimmunized hens treated in parallel in the chloroform step (crude IgG) and then in precipitation with $(NH_4)_2SO_4$ (precipitated IgG). The IgG preparations were from eggs produced 18 and 19 days after booster injection.

[b] Mean value for at least two wells for each treatment following 45 min reaction at room temperature in enzyme immunosorbent assay (EIA) in test of unreacted virus from previous absorption reaction.

[c] No. of plants infected, of 12 infested, when *Schizaphis graminum* acquired virus by feeding through membranes on same SGV samples shown at left.

undiluted, 1:2, 1:4, and 1:8 dilutions, respectively. The assay seemed adequate at least for detecting large differences in antibody titer among the preparations.

In nine experiments the mean absorbance value for MAV previously incubated with control solutions was 1.083 (range, 0.473–1.434) for saline and 1.095 (range, 0.452–1.476) for normal yolk preparations. When anti-MAV immunoglobulin was used as another control, no unreacted virus was detected (mean value of −0.001). Incubation of MAV with each of the 57 crude IgG preparations did not remove all of the virus, but it did consistently remove most of it. The mean absorbance value for MAV previously incubated with crude IgG preparations was 0.091 (range, 0.006–0.311). Results were surprisingly consistent among the nine experiments. In tests of 50 of the 57 preparations, individual EIA values were from 1 to 10% of the corresponding value of each normal yolk control in the same experiment. Only seven of the values were greater than 10% of the control; most of these occurred in one experiment. Some of the same crude IgG preparations from early experiments were included in all later tests to have some means of relating one titration experiment with another. Consistency among these comparisons provided additional evidence that these crude titrations were useful despite their heterologous basis. For example, the absorbance values for one preparation (from eggs obtained 86–90 days after the booster injection) in each of eight succeeding experiments during the 15-mo period were 5, 4, 3, 1, 2, 2, 7, and 2% of each respective yolk control. These data are in agreement with observations of others (15,24) that antibody titer in hen egg yolks remains reasonably stable for a long period following immunization.

Although most crude IgG preparations were made with chloroform (13), we compared this procedure with the PEG method (15). We selected 16 eggs produced between 269 and 294 days after the booster injection, divided them into four groups of four each, and used them to make two preparations by each method. We found no differences among these four IgG preparations in any of several kinds of comparisons. For example, in absorption tests with MAV, the mean absorbance values for unreacted MAV were 0.027 and 0.021 for the two preparations made with chloroform; corresponding values were 0.026 and 0.027 for the preparations made with PEG. None of the preparations was active in the coating step of EIA; all gave equivalent reactions in indirect EIA.

We confirmed the presence of SGV antibodies in these crude IgG preparations in another kind of experiment by incubating IgG with purified SGV and measuring the amount of unreacted virus after sucrose gradient centrifugation. We incubated (37 C for 30 min; 4 C overnight) 14 μg of SGV with saline and with crude IgG preparations made from eggs collected 26–28 days and 134–135 days after the booster injection. Centrifugation was for 3.5 hr on rate-zonal sucrose gradients (20). Reactions with both IgG preparations removed essentially all SGV, but none was removed by the saline. In a bioassay of virus zones from the gradients, no virus was transmitted by *S. graminum* fed on zones from IgG preparations, but only two transmissions occurred from the saline control zone. Tests with 1:10 dilutions of these IgG preparations provided some evidence that the antibody titers of the 57 preparations was not necessarily as similar as our absorption tests with MAV indicated. The 1:10 dilution of the 26- to 28-day preparation had removed most of the 14 μg of SGV, but the same dilution of the 134- to 135-day preparation removed only about 6 μg. This also indicates that the actual antibody titer in these preparations is relatively low; antisera produced in rabbits against other isolates of BYDV absorb virus in similar tests at dilutions of 1:1,000 or more.

TABLE 2. Comparison of precipitated and purified anti-SGV IgG in the coating step of direct enzyme immunosorbent assays

Dilution of IgG[a]	Absorbance at 405 nm in reaction with SGV and IgG preparation shown[b]	
	Precipitated	Purified
1:10	0.010	0.545
1:50	0.044	0.517
1:250	0.064	0.425
1:1,250	0.078	0.198
1:6,250	0.082	0.044
1:31,250	0.021	0.004
Anti-MAV IgG (10 μg/ml)	0.430	0.249
Precipitated IgG (5 μg/ml)	-	0.087

[a]The precipitated IgG was diluted from a preparation containing 2.0 mg/ml; the purified IgG was diluted from a preparation containing 0.56 mg/ml of protein.

[b]Data are means from two separate tests using enzyme labeled precipitated IgG, a substrate reaction of 45 min at room temperature, and a 30-fold concentrate of SGV. The mean of all "healthy controls" was 0.001.

Use of anti-SGV IgG in EIA. We made many attempts to use both crude and precipitated IgG in direct EIA tests for SGV, but only weak, inconsistent reactions were obtained. To study possible improvements, we tested various dilutions of IgG and different SGV preparations, including some that were concentrated and partially purified; different incubation times and different combinations of temperatures for the various steps; both polyvinyl chloride and polystyrene plates; and phosphate buffers at pH 6–8 and carbonate-bicarbonate buffers at pH 9–10 to study a pH range for both the coating and antigen incubation steps. None of these variations produced reactions stronger than those done under our standard conditions. Absorbance values were almost always less than 0.1. In all tests, reactions in control wells coated with anti-MAV immunoglobulins were stronger than those in wells coated with anti-SGV IgG.

We did not study the basis for difficulties in using precipitated IgG in the coating step, but they could be due to presence of other yolk components that act as inhibitors in the reaction between coating antibody and virus. This was suggested by observations that more dilute samples of precipitated IgG often were more effective in coating than less dilute ones (Table 2). Although precipitated IgG was not useful in the coating step, purified IgG worked well with 30-fold concentrates of SGV (Table 2). Moreover, reactions of dilutions of purified IgG suggest that inhibitors were not present after purification (Table 2). Since the purified IgG was produced near the end of these studies, we have not made extensive tests with it.

These putative inhibitors may not prevent IgG coating of the wells but reduce interaction of coating antibody and virus. When we coated wells with precipitated IgG, used rabbit anti-chicken globulins in the second step, and finally applied goat anti-rabbit conjugated globulin, we observed strong reactions (absorbance values from 0.405 to 0.750). This suggests that immunoglobulins from yolk can actually coat a well. When we used rabbit anti-chicken globulin to coat wells, and then reacted our precipitated IgG next, we obtained very weak reactions.

Although we could not use precipitated IgG in direct EIA tests, we could routinely use it in an indirect EIA, an experience similar to that of Hsu and Lawson (8). The indirect procedure used a monoclonal antibody to coat EIA plates. This antibody (mAB-MAV4), produced against MAV, reacts with both MAV and SGV (7). Optimum dilutions for reactants were found to be 1:1,000 for mAB-MAV4, 1:800 (2.5 μg/ml) for precipitated IgG, and 1:300 for the commercial conjugate. This indirect EIA permitted detection of SGV in clarified preparations and in partially purified preparations at concentrations as low as 15 ng/ml (Fig. 1). In parallel tests (same plate) with SGV, MAV, and PAV (trapped by rabbit anti-PAV IgG), homologous reactions with SGV were consistently stronger than those with MAV and PAV (Fig. 1). Since mAB-MAV4 only reacts with MAV and SGV, and not with PAV, this indirect test made it possible to assay SGV in the presence of PAV (Table 3). Results of other tests showed that this indirect EIA could also be used to assay SGV in the presence of MAV if antigen preparations were first absorbed with mAB-MAV1, a monoclonal antibody that reacts only with MAV (7).

Serological relationship of SGV to other barley yellow dwarf viruses. Results of five kinds of experiments consistently showed that SGV was related to MAV and PAV, but distinct from RPV and RMV. One line of work was based on absorption reactions

carried out in test tubes. Clarified virus preparations were mixed with crude IgG-A, crude IgG-B, a preparation from normal egg yolks, and saline; and then assayed in two ways. Unreacted virus was measured in direct EIA tests with homologous immunoglobulins; parallel tests were made of the same samples by allowing appropriate aphid vectors to feed on them. In every experiment results with the two crude IgG preparations were the same, as were results with the two kinds of controls. Results of these parallel treatments were combined to calculate one mean value for each of the treatments and for the controls, as summarized in Table 4. In early experiments with SGV, values from both kinds of assays were very low when clarified SGV preparations were used. In several additional tests, we used 30-fold concentrates of SGV (Table 4). Results of both assays showed SGV to have reacted completely in absorption tests. We always used each virus preparation at two dilutions because sensitivities of the two assays used to evaluate the absorption were different for some viruses. In experiments with MAV, for example, the aphid transmission tests were more sensitive than EIA and diluted virus preparations were needed to observe any possible difference in virus transmission assays (Table 4). Results of tests with MAV and PAV were similar to those with SGV. Both crude IgG preparations reacted completely with virus and reduced or eliminated transmission by the appropriate aphid vector. In contrast, the anti-SGV IgG had no effect on RPV or RMV in either kind of test (Table 4).

When the indirect EIA assay was developed for SGV, it provided another way to compare the five viruses. In one series of experiments each virus was trapped in EIA plates by its homologous immunoglobulin, except for SGV, where mAB-MAV4 was used for coating. We used precipitated IgG in reactions with the trapped viruses and then used labeled rabbit anti-chicken globulin to measure the reaction. In each of four experiments, anti-SGV IgG reacted with SGV, MAV, and PAV, but not with RPV or RMV (Table 5). In three of these four experiments reactions with PAV and MAV were actually stronger than those with SGV, an illustration of the relative concentrations of the three antigens in clarified preparations (Table 5). In two additional experiments, we used the same immunoglobulins for trapping each virus, but used labeled, precipitated IgG to carry out a direct assay in place of an indirect one. Results were similar to those from the indirect comparison; anti-SGV IgG reacted with PAV, MAV, and SGV, but not with RPV or RMV (Table 5).

Results of micro agar double-diffusion tests also showed that

TABLE 3. Detection of SGV in the presence of PAV by the indirect enzyme immunosorbent assay

Antigen tested	Coating antibody	Absorbance at 405 nm[a]
SGV	mAB MAV-4	0.682
SGV + PAV	mAB MAV-4	0.414
PAV	mAB MAV-4	0.000
PAV control	PAV-Ig	0.577
Saline control	mAB MAV-4	−0.003
Healthy control	mAB MAV-4	0.000

[a] Precipitated anti-SGV IgG (2.5 μg/ml) was reacted with trapped virus and the reaction was measured with rabbit anit-chicken immunoglobulin conjugate following incubation with substrate for 45 min at room temperature. Values are means of two wells for each treatment.

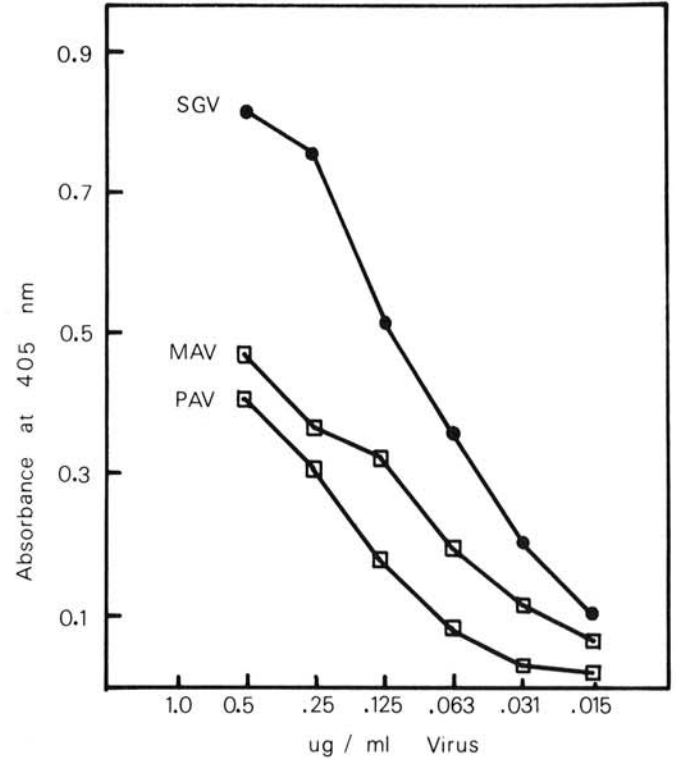

Fig. 1. Reaction of purified preparations of the SGV, MAV, and PAV isolates of barley yellow dwarf virus with anti-SGV IgG in indirect enzyme immunosorbent assays. The monoclonal antibody MAV-4 was used to trap SGV and MAV; PAV was trapped with anti-PAV immunoglobulin from rabbits. The chicken IgG reacted with trapped virus; labeled rabbit anti-chicken conjugate was used to evaluate the reaction. The enzyme reaction was measured after 45 min at room temperature.

TABLE 4. Relationships of five isolates of barley yellow dwarf virus with anti-SGV hen IgG in absorption reactions evaluated in two ways

Virus and dilution tested[a]	Absorbance at 405 nm in direct enzyme immunosorbent assay (EIA) after absorption with crude IgG[b]		Aphids used as vectors	Infectivity of virus preparation after absorption[c]	
	SGV IgG	Normal IgG		SGV IgG	Normal IgG
SGV 1:1	0.001(±0.000)[d]	0.131(±0.022)	*Schizaphis graminum*	0(±0.0)[d]	12(±0.5)
SGV 1:5	−0.002(±0.000)	0.106(±0.007)		0(±0.0)	12(±0.9)
MAV 1:1	0.006(±0.000)	0.280(±0.016)	*Sitobion avenae*	6(±1.0)	11(±0.4)
MAV 1:10	−0.001(±0.001)	0.012(±0.002)		1(±0.8)	10(±0.8)
PAV 1:1	0.001(±0.001)	0.461(±0.090)	*Rhopalosiphum padi*	9(±1.3)	11(±0.6)
PAV 1:10	−0.003(±0.001)	0.008(±0.002)		3(±0.3)	8(±1.4)
RPV 1:1	0.222(±0.056)	0.213(±0.054)	*R. padi*	9(±0.9)	11(±0.8)
RPV 1:10	−0.023(±0.006)	−0.027(±0.006)		10(±1.5)	10(±1.0)
RMV 1:1	0.066(±0.013)	0.070(±0.015)	*R. maidis*	9(±1.3)	9(±1.5)
RMV 1:10	−0.013(±0.001)	−0.013(±0.002)		6(±1.5)	7(±1.5)

[a] The identity and reactivity of each virus preparation was confirmed in direct EIA with four virus-specific immunoglobulins.
[b] Mean values of eight wells (two experiments) following 45 min of reaction at room temperature with homologous immunoglobulins except for SGV, where heterologous anti-MAV immunoglobulin was used. Mean value of all healthy controls was 0.006.
[c] Mean of four values from two experiments. None of 120 plants infested as controls became infected.
[d] Numbers in parentheses are standard errors.

•SGV was related to MAV and PAV, but distinct from RMV and RPV. When precipitated IgG preparations were first absorbed with concentrates of healthy oats (22) and used in agar diffusion tests along with rabbit immunoglobulins, reciprocal precipitation lines formed in several tests with SGV, MAV, and PAV, but not in similar tests with RPV and RMV. Relative concentrations of antibody and virus did not allow a study of spur formations and other detailed aspects of the relationships among the viruses.

Test of dependent transmission. Since the indirect EIA provided a means to identify SGV in the presence of PAV (Table 3), we used this assay to determine if *R. padi* could transmit SGV from plants doubly infected with PAV, despite its inability to transmit SGV from singly infected plants. This mixed infection of SGV and PAV was the only combination not studied in a previous investigation of dependent virus transmission among mixed infections of the five viruses (18). In each of three experiments, we found that *R. padi* did not transmit SGV from plants also infected with PAV, nor did it transmit SGV from any singly infected controls (Table 6). Although PAV is a helper virus for the dependent transmission by *R. padi* of both RMV and MAV (18), it does not also serve as a helper virus for the dependent transmission of SGV.

DISCUSSION

These results are in agreement with findings from studies of other viruses that show some advantages of chickens over other animals for producing virus-specific antibodies. We obtained a large volume of antibody preparations, antibody titer in the hen appeared to remain relatively constant during a long period of time, procedures for isolating antibodies from egg yolks were simple, and the antibodies were especially useful in indirect EIA with immunoglobulins from rabbits or mice. Our data on the apparent similarity of antibody titer among the 57 preparations made during the 15 mo of this work are clouded by the relatively crude nature of the absorption reaction with heterologous (MAV) antigen, and by results of one test using purified SGV. We conclude that the titers among the preparations were relatively similar, but that titers were low.

The low titer was probably one of three important factors that prevented use of the IgG in the coating step of direct EIA assays. Low antibody titer was suggested by the low dilutions needed to react with 14 μg of purified SGV and by results of agar diffusion tests. It was also suggested by our yield of purified IgG. We obtained only about 5 mg/ml of protein; Polson et al (15) had yields of 6–12 mg/ml. This low titer is not surprising in view of the small amount of virus injected into the hen. The hen we immunized received less than 100 μg of SGV in a total of two injections. Polson et al (15) used 1–5 mg of virus for each of several injections.

The second factor that probably affected our coating problem was the presence of other yolk components that acted as inhibitors. The third factor appeared to be low concentration of SGV in clarified preparations usually used. We routinely obtained strong reactions in direct EIA with all four of the other BYDV isolates in clarified preparations, but we got similar strong reactions for SGV only when we used about 30-fold concentrates of virus. Another possibility, suggested by Hsu and Lawson (8), is that failure of the chicken antibodies in the coating step is due to inaccessibility of the immobilized antibodies to antigenic determinants on the virions. Despite limitations, the chicken antibody has already proved useful in a variety of tests, and we think that use of purified IgG and improvements in the indirect EIA procedure will expand possibilities.

Results of all five kinds of assays on serological relationship of SGV to the other four virus isolates were in clear agreement. This work extends previous serological studies (with immunoglobulins against MAV, PAV, RPV, and RMV) that showed SGV to be related to PAV and MAV, but distinct from RPV and RMV, which in turn are related to each other. Separation of these viruses into the two groups is now supported by serological data (19,22), by cytological studies (5), by analysis of double-stranded RNA from infected plants (3), and by comparative studies of nucleic acid relatedness of the viruses (M. Zaitlin and P. Palukaitis, *personal communication*). This grouping should provide a basis for future improvements in nomenclature of these virus isolates, which also are related to other luteoviruses responsible for yellows diseases of many crops (23).

The chicken IgG was especially useful in studies of dependent virus transmission by aphids from mixed infections. In dependent transmission, aphids transmit one virus (dependent virus) only in the presence of a second virus (helper virus). This interaction, which appears to be a special feature of plant virus transmission by aphids, is a major focus of work at Cornell to understand relationships between these luteoviruses and their aphid vectors. In the past we have investigated the potential for dependent virus transmission for 14 combinations among the five virus isolates and four aphid species (18). But we could not study the interaction of PAV and SGV because we had no way to identify SGV in the presence of PAV. Tests described here show that PAV is not a helper virus for dependent transmission of SGV. Thus, dependent virus transmission occurs for seven of the 15 possible virus interactions. Since PAV does serve as a helper virus for the dependent transmission of MAV by *R. padi*, we were somewhat

TABLE 5. Relationship of SGV to other isolates of barley yellow dwarf virus determined with anti-SGV hen IgG in indirect and direct enzyme immunosorbent assays (EIA)

Virus[a]	Coating antibody[b]	Absorbance at 405 nm in kind of EIA shown[c]	
		Indirect	Direct
RPV	RPV-Ig	0.011 (±0.003)	0.004 (±0.003)
RMV	RMV-Ig	0.007 (±0.002)	0.002 (±0.001)
PAV	PAV-Ig	0.876 (±0.142)	0.139 (±0.030)
MAV	MAV-Ig	0.820 (±0.133)	0.144 (±0.022)
SGV	mAB-MAV4	0.495 (±0.080)	0.122 (±0.030)

[a]The identity of each virus preparation was confirmed in direct EIA with four virus-specific immunoglobulins. That both RPV and RMV were trapped by each homologous immunoglobulin was shown by mean absorbance values of 0.955 and 0.279, respectively.

[b]Homologous immunoglobulins were used for all but SGV, for which the monoclonal antibody shown was used in the coating step.

[c]Values are means from four experiments for indirect assays and from two experiments for direct assays. In each experiment values were means of two wells following 45 min of reaction at room temperature. In the indirect assays, the second antibody was precipitated anti-SGV IgG (diluted 1:800) and the conjugate was rabbit anti-chicken immunoglobulin diluted 1:300. In the direct assays, the conjugate was labeled, precipitated IgG diluted 1:100. Numbers in parentheses are standard errors.

TABLE 6. Tests for dependent transmission of SGV by *Rhopalosiphum padi* in the presence of PAV

Transmission by *R. padi* from plants singly infected with SGV (S) or doubly infected with SGV and PAV (D)[a]			Absorbance at 405 nm in enzyme immunosorbent assays (EIA) to identify viruses transmitted by *R. padi* from each doubly infected plant[b]	
SGV(S)	SGV(D)	PAV(D)	SGV	PAV
0	0	12	0.009 (±0.002)	0.903 (±0.040)
0	0	12	0.010 (±0.005)	0.754 (±0.048)
0	0	12	0.004 (±0.001)	0.770 (±0.040)

[a]Number of plants (of 12) found to be infected by virus shown. In parallel tests with *Schizaphis graminum*, SGV was transmitted from all 36 singly infected cultivar Coast Black oat plants and from 35 of 36 doubly infected ones.

[b]Data are means of 12 values for each of the three experiments. Identification of PAV was based on direct EIA with PAV-immunoglobulins; SGV was identified in indirect EIA using mAB MAV-4 to trap virus, which was reacted with precipitated anti-SGV IgG. Each sample was tested in at least two wells; enzyme reactions were for 45 min at room temperature. Mean value for SGV from controls infected by means of *S. graminum* was 0.468. Mean value of all healthy controls was 0.009. Numbers in parentheses are standard errors.

surprised to find that it does not have a similar function for the dependent transmission of SGV by *R. padi*, especially since RPV is a helper virus for the dependent transmission of SGV by *R. padi*. This finding is another example of the remarkable specificity among the viruses and vectors of this system, a feature of the virus-vector interactions we have encountered in many kinds of tests in the past. It may be significant that four of the examples of dependent virus transmission occur between distinct virus isolates from each of the two groups. Only the dependent transmission of MAV in the presence of PAV, and the one case of reciprocal virus transmission for mixed infections of RPV and RMV, involve the interaction of related viruses. These examples now provide a broad base for detailed study of the mechanism for dependent virus transmission.

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