Development of a Solid-Phase Radioimmunoassay for Detection of Soybean Mosaic Virus

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ABSTRACT

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A direct sandwich solid-phase radioimmunoassay (SPRIA), using antibody-coated polystyrene beads, was developed for the detection and quantification of soybean mosaic virus (SMV) in soybean seeds. Virus-specific IgG was passively adsorbed to the solid-phase bead surface, and virus antigen, bound by the immobilized antibody, was detected by subsequent binding of anti-SMV IgG labeled with ^{3}H. SPRIA values (or bound radioactivity) were generally proportional to virus concentration with a detection limit of 25–50 ng of purified SMV per milliliter. Extracts from healthy seeds did not interfere with virus detection. Extracts from infected and uninfected seeds could be differentiated, and the approximate level of infection could be estimated by using SPRIA. Because of its technical simplicity, reproducibility, and versatility, SPRIA is useful for detecting SMV in individual and bulked seeds.

Additional key words: seed transmission, serology.

Soybean mosaic virus (SMV), a member of the potyvirus group of plant viruses (13,14), is one of several seed-transmissible pathogens in soybean (28) that may lead to significant yield losses (34) and reduced seed quality (8). Since its first reported appearance in Connecticut, the mosaic disease has spread until it now coexists with soybean cultivation almost worldwide (3). This wide geographic range is directly attributed to commercial distribution and planting of infected seed. Secondary spread occurs by aphids in a nonpersistent manner (25). The incidence of soybean mosaic throughout major soybean-producing areas and the ease with which it is introduced into new areas emphasizes the need for

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suitable screening procedures, especially to certify the virus-free status of breeder's seed and commercial seed shipments. We describe details of a quantitative radioimmunoassay used in field studies to detect SMV antigen in seed (5). With a reliable test for virus detection, quantitative estimates of virus content in seed lots should provide seed producers with a means for assessing the advisability of distributing seed lots containing SMV-infected seed into the commercial market.

MATERIALS AND METHODS

Virus. One isolate from each of the seven SMV strains (G1–G7) identified by Cho and Goodman (7) and Ia 75-16-1 (13,14) were purified from infected *Glycine max* 'Williams' soybeans as described previously (13,15). Concentrations were estimated spectrophotometrically by using $E^{0.1\%}_{260\,nm} = 2.4$ (31).

Immunoglobulin G (IgG) purification. Anti-SMV-IgG was purified from hyperimmune serum produced in rabbits as described by McLaughlin et al (26). Titer of the antibody preparation used in these experiments was 1:256 (undiluted IgG concentration = 4.4 mg/ml as determined by $E^{0.1\%}_{280} = 1.4$) when tested in microprecipitin tests against an antigen concentration of 0.5 mg of purified SMV per milliliter.

Tritium labeling of IgG. Procedures to label lyophilized anti-SMV-IgG were as described by Rifkin et al (32) except that 100 mCi of NaB^3H_4 (specific activity 10–20 Ci/mmole, Research Products International Corp., Elk Grove Village, IL 60067) were added sequentially in four steps. A 5-min incubation at 0–4 C followed the first three additions and a 15-min incubation terminated the sequence. Free ^3H was removed by passage of the reaction mixture through a 3 × 33-cm Sephadex G25 (Pharmacia Fine Chemicals, Piscataway, NJ 08854) column equilibrated in 0.05 M sodium borate, pH 7.2, containing 0.85% NaCl (BBS) and presaturated with 1% bovine serum albumin (BSA) in BBS. Fractions constituting the labeled immunoglobulin protein peak were pooled, dispensed in small volumes, and stored at −20 C. Specific activities of various batches ranged from 8 to 38 μCi/mg protein.

Specificity of ^{3}H-anti-SMV-IgG. A competitive radioimmunoprecipitation (RIP) binding assay (21) was performed to demonstrate the specificity of the assay and eliminate the possibility that the reaction was caused by nonspecific binding of ^{3}H-IgG to the virus surface. Increasing amounts of unlabeled homologous or heterologous antibodies were incubated with a constant saturating amount of labeled antibody (5–15 μg) in a total volume of 1.0 ml for 1 hr at 37 C and then overnight at 4 C. After centrifugation at 20,000 *g* for 20 min at 4 C, 0.2-ml quantities of the supernatants were counted. Virus-bound radioactivity was calculated from the formula: $\%T = (S_c - S_t/S_c)100$ in which $\%T$ = percentage of total input activity bound by the virus, S_t = cpm/0.2 ml of supernatant from tubes containing virus, and S_c = cpm/0.2 ml of supernatant from a control tube without virus. Supernatants rather than precipitates were used for counting because the flocculant precipitates could not be resuspended evenly. Heterologous antibody sources were antisera to TMV and SBMV. Inhibition of the reaction between ^{3}H-anti-SMV-IgG and virus by unlabeled antibody was determined by comparing percentage binding in the presence of competing antibody, with maximum binding in the absence of competing antibody.

The amount of labeled antibody immunologically precipitable by the virus was determined by an RIP assay. Various amounts of SMV were incubated with 5 or 15 μg ^{3}H-anti-SMV-IgG in a total volume of 1.0 ml as described.

Radioimmunoassay. The bead enzyme-linked immunoassay (ELISA) (36) was modified for use in a comparable SPRIA for SMV. Polystyrene "frosted" beads (3.4 mm in diameter; Precision Plastic Ball Co., Chicago, IL 60641) were adsorbed in a solution of anti-SMV-IgG diluted in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.6 (coating buffer). Groups of 100 beads were coated for 20 hr at room temperature by gentle rotation at a protein concentration of 50 μg/ml. The coating solution was aspirated, and the beads were washed three times (1 ml/bead/3–5 min wash) with 0.02 M sodium-potassium phosphate buffer, pH 7.3, containing 0.8% NaCl, 0.05% Tween-20 (Sigma Chemical Corp., St. Louis, MO 63178) (PBS-Tween), 2% polyvinylpyrrolidone (PVP, mol wt 40,000; Sigma Chemical Corp.), and 0.2% ovalbumin (OVA, Grade II; Sigma Chemical Corp.) to remove nonadsorbed or loosely bound IgG. Beads were incubated 1 hr longer in the buffer and were air-dried at room temperature and stored at 4 C.

To assay virus and seed extracts, sensitized beads were dispensed individually into 12 × 75-mm plastic tubes (Falcon Plastics, Los Angeles, CA 90045) precoated 1–2 hr with 2% OVA in PBS-Tween, and rinsed three times with PBS-Tween. Test samples (0.5 or 1.0 ml), either diluted in 0.05 M sodium borate buffer (BB) (pH 7.2) for seeds or in BB containing 0.5% sodium metaphosphate $(NaPO_3)_{13}$ for purified virus, were incubated with the solid-phase antibody for 20 hr at room temperature with gentle agitation. The samples were aspirated, and the beads were washed as before. Beads were transferred to clean precoated plastic tubes and 200 μl of ^{3}H-anti-SMV-IgG in PBS-Tween containing 2% OVA was added to each bead at a predetermined optimal concentration obtained by calculation of binding ratios (15). The reactants were gently agitated at room temperature for 8 hr and the assay was terminated by aspiration of the labeled antibody. The beads were washed with PBS-Tween-PVP-OVA and placed into 5.0 ml of Riafluor scintillation cocktail (New England Nuclear, Boston, MA 02118) for measurement of bound ^{3}H-anti-SMV-IgG in a Beckman DPM-100 liquid scintillation system with a counting efficiency of 46.7% for ^{3}H. Reactions were regarded as positive when bound radioactivity was greater than the mean of the control (containing no virus antigen) plus three standard deviations. Details of experiments to examine parameters in SPRIA development are presented in the Results section.

The specificity of the assay was determined by comparing reactivity to the SMV isolates from strains G1–G7; maize dwarf mosaic virus, strain A (MDMV-A); southern bean mosaic virus (SBMV); cowpea mosaic virus, strain Sb (CPMV-Sb); and tobacco mosaic virus (TMV) with that of the homologous system.

Extraction of seeds for SPRIA. Batches of 100 seeds were soaked for 6–12 hr in 100 ml of BB and homogenized for 1 min at setting 6 in a Brinkman polytron homogenizer equipped with a model PT 20 ST probe generator (Brinkman Instruments, Inc., Westbury, NY 11590). Extracts were squeezed through cheesecloth; 1.0-ml subsamples were assayed for SMV by SPRIA.

Seeds from a lot of the cultivar Williams with a transmission rate of approximately 54%, based on visual inspection of seedlings, were soaked in water, dissected into halves along the embryonic axis, and allowed to dry. Half of each seed was powdered with mortar and pestle and extracted overnight at 4 C with 0.5 ml of BB. An antibody-coated bead was added to each extract, and the assay was completed as described earlier.

Greenhouse experiments. Seeds of the cultivar Amsoy 71 were sown in a soil mix in 10-cm-diameter clay pots, thinned to four per pot after emergence, and fertilized with a 14-14-14 slow-release fertilizer. Treatments consisted of plants mechanically inoculated with SMV (Ia 75-16-1) at growth stage V-1 (9) with 0, 5, 10, 20, 30, 40, 60, 80, and 100% of the plants in each treatment inoculated. Each treatment consisted of 20 pots each replicated eight times with the treatments randomized within each replication. Seeds from each treatment were harvested, dried to uniform moisture, and weighed. The SMV antigen content of batches of 100 seeds from each treatment was determined by SPRIA.

Detection of SMV in aphids. Apterous adult green peach aphids, *Myzus persicae* Sulzer, were starved for 2–3 hr and then given a 5- to 10-min acquisition access to detached SMV-infected cultivar Williams soybean leaves. Groups of 5, 25, 50, and 65 aphids then were triturated in 0.5 ml BB containing 0.5% $(NaPO_3)_{13}$ and assayed for virus acquisition by SPRIA. Equal numbers of nonviruliferous aphids given access to healthy leaves were used as controls.

RESULTS

Characterization of ^{3}H-anti-SMV-IgG. Labeling of IgG with

tritium had no deleterious effect on antibody quality as assessed by SDS-polyacrylamide gel electrophoresis (16); Sephadex gel chromatography; microprecipitin titers (13) of pre- and postlabeled IgG; and immunodiffusion patterns (17) with goat anti-rabbit gamma globulin (Kallestad Laboratories, Minneapolis, MN 55318), normal rabbit IgG (Sigma Chemical Corp., St. Louis, MO 63178), anti-SMV-IgG, and ^{3}H-anti-SMV-IgG (*unpublished*).

Specificity of the reaction was demonstrated by RIP. When a constant amount of ^{3}H-anti-SMV-IgG was added to various amounts of SMV, about 65% of the total radioactivity was bound at saturation (Fig. 1A), demonstrating that a major portion of the immunological activity was retained. The binding of radioactivity was virus-specific because increasing amounts of unlabeled homologous antibody competitively inhibited the binding of labeled antibody, but unlabeled heterologous antibody had little effect (Fig. 1B).

Development of SPRIA. Investigation of the rate of antibody adsorption to the solid phase showed that with 10 μg of available ^{3}H-anti-SMV-IgG per bead, only 1% of the total input radioactivity was bound during a 16-hr adsorption period. After 3 hr, 96% of the total protein bound was adsorbed. An adsorption period of approximately 20 hr was chosen, however, for routine use as a matter of convenience and because prolonged adsorption may allow more uniform coating.

Binding of ^{3}H-IgG could be blocked by 64 and 81%, respectively, when either 10 or 33 μg of IgG was added before addition of ^{3}H-IgG (10 μg). This could be important when crude serum, containing numerous proteins, is used for the initial coating.

Nonspecific reactions can obscure differences in specific binding or contribute to false positive readings. Various buffer additives, including PBS-Tween containing either 0.2% OVA, 2.0% PVP, 0.2% bovine serum albumin, 0.2% gelatin, 0.2% normal rabbit serum, or 0.2% fetal calf serum reduced nonspecifically bound ^{3}H-anti-SMV-IgG to less than 1% of the total input activity; the inclusion of various proteins into the PBS-Tween buffer reduced nonspecific binding from 44% (PBS-Tween-PVP-OVA) to 61% (PBS-Tween-OVA) of the PBS-Tween control.

The initial concentration of anti-SMV-IgG coating antibody determined the amount of antibody bound to a bead until saturation of potential binding sites was achieved. An antibody protein concentration of 50 μg/ml was optimal (Fig. 2). The polystyrene beads could be dried and stored at 4 C for at least 3 mo with no appreciable change in binding activity.

Effects of temperature (4, 28, and 37 C) and incubation time (1, 2, 4, or 20 hr) on binding of viral antigen (1,000 ng/ml) to immobilized antibody (10 μg per bead) were investigated. Maximum binding occurred at 28 C for 20 hr. Similarly, reaction of the solid-phase components with ^{3}H-anti-SMV-IgG showed comparable binding at 28 and 37 C and reduced binding at 4 C. Binding increased linearly during the first 2–4 hr of incubation and then leveled off. An 8-hr incubation with gentle shaking at room temperature was adopted so that the secondary reaction could be completed within a working day.

Dilution of SMV in PBS-Tween frequently caused spontaneous precipitation of the virus and erratic or reduced binding. The addition of 2% PVP alone or in combination with 0.2% ovalbumin decreased binding, and erratic binding occurred when BB was used

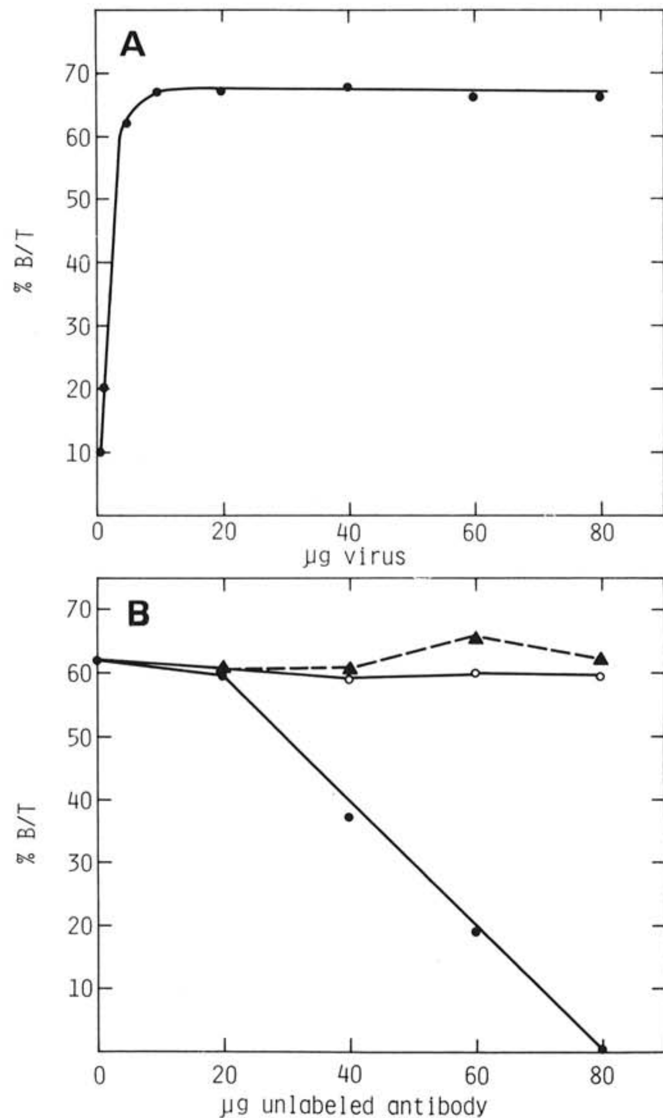

Fig. 1. Radioimmune precipitation of ^{3}H-anti-soybean mosaic virus (SMV) IgG by virus. **A,** Homologous binding of ^{3}H-anti-SMV-IgG by SMV. The reaction mixture contained 5 or 15 μg of labeled antibody with increasing amounts of SMV in a total volume of 1.0 ml. Results are expressed as percentage of total radioactivity bound (B/T) by the virus. **B,** Specificity of the binding reaction between ^{3}H-anti-SMV-IgG and SMV. Each assay contained 5 or 15 μg of labeled antibody and 5 or 20 μg of SMV to which increasing amounts of unlabeled homologous (●——●) or heterologous (anti-tobacco mosaic virus, O——O; anti-southern bean mosaic virus, ▲——▲) antibody were added.

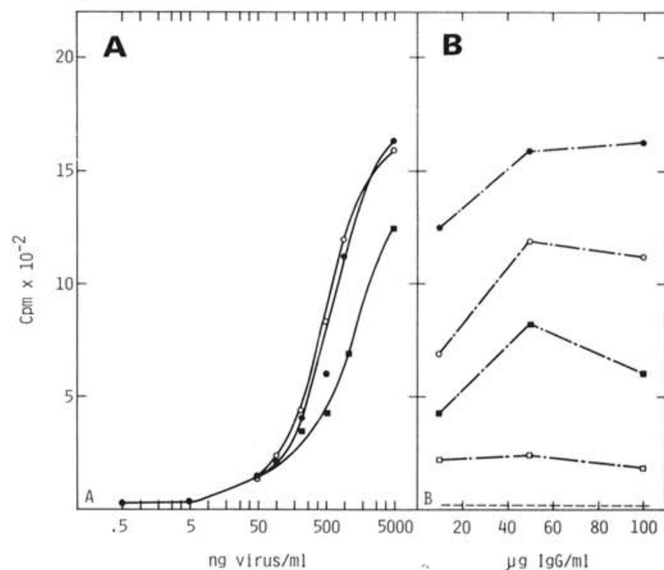

Fig. 2. Effect of protein concentration on adsorption of anti-soybean mosaic virus (SMV) IgG on polystyrene beads. Beads adsorbed for 20 hr at each IgG concentration were incubated with SMV for 20 hr and ^{3}H-anti-SMV (1 μg/ml) for 8 hr. Cpm (counts per minute) bound versus virus concentration when beads are adsorbed with: **A,** 10 μg IgG per milliliter (■——■), 50 μg IgG per milliliter (●——●), 100 μg IgG per milliliter (o——o). **B,** Cpm bound versus IgG coating concentration for virus concentrations of: 250 ng SMV per milliliter (□——□), 500 ng SMV per milliliter (■——■), 1,000 ng SMV per millilter (o——o), 5,000 ng SMV per milliliter (●——●), buffer control (------).

as antigen diluent. The dilution of infected seed extract in BB, however, resulted in reproducible binding as did dilution of purified virus in extracts of healthy seeds prepared in BB. This suggested that, if erratic binding was caused by virus aggregation, it could perhaps be circumvented with a suitable diluent. SMV was added to different buffers, mixed for 1 min with a Brinkman polytron homogenizer to simulate the homogenization used in preparation of seed samples for assay, and assayed by SPRIA. The highest binding activity was obtained with SMV in either healthy seed extracts, healthy extracts containing 0.5% $(NaPO_3)_{13}$, or borate containing 0.5% $(NaPO_3)_{13}$ (Table 1). Seeds were extracted in borate alone because nonspecific binding was high with seeds extracted in borate containing $(NaPO_3)_{13}$. In tests with purified virus, 0.5%, $(NaPO_3)_{13}$ was included in the diluent to reduce virus aggregation.

TABLE 1. The effect of borate buffer additives on binding[a] of purified soybean mosaic virus in solid-phase radioimmunoassay

Buffer	Cpm bound[a] from a virus concentration (ng/ml) of:				
	5,825	2,913	1,457	728	0
BB[b]	164	83	218	58	42
BB + 0.05% soybean oil	79	68	792	46	42
BB + 0.05% soybean oil + 2% polyvinylpyrrolidone (mol. wt. 40,000)	117	135	102	68	37
BB + 0.5% Triton X-100	117	258	87	63	43
BB + 0.5% sodium dodecyl sulfate	46	43	39	44	38
BB + 0.5% Nonidet P-40	251	136	179	118	44
BB + 0.5% $(NaPO_3)_{13}$	3,151	2,379	2,146	1,105	38
HSE	2,977	2,707	1,771	1,698	53
HSE + 2% polyvinylpyrrolidone (mol. wt. 40,000)	510	544	215	89	65
HSE + 0.5% $(NaPO_3)_{13}$	2,580	1,836	2,084	1,597	145

[a] Activity measured as cpm (counts per minute) bound under standard assay conditions.
[b] BB = 0.05 M sodium borate, pH 7.2; HSE = healthy seed extract (1:10 w/v in BB).

The optimal concentration of ^{3}H-IgG for maximum antigen detection ranged from 1 to 5 μg of protein per milliliter, depending on the labeled preparation examined. Adequate washing of the solid phase was essential to remove traces of labeled reactants. Replicate IgG-coated beads incubated 8 hr with ^{3}H-anti-SMV-IgG before washing them either 0, 1, or 3 times with PBS-Tween-PVP-OVA showed less than 0.5% of the total radioactivity in the incubation medium was nonspecifically bound, indicating successful blocking of potential unfilled protein-binding sites. After one wash, this was reduced to approximately 0.36%. Further washes had no additional effect. Similar results were obtained when beads were incubated with virus before the addition of labeled antibody.

Detection of SMV. Purified SMV was detected to levels of approximately 25–50 ng/ml under optimal assay conditions (Fig. 3). The relationship between virus concentration and counts per minute (cpm) bound generally was stoichiometric between 250 and

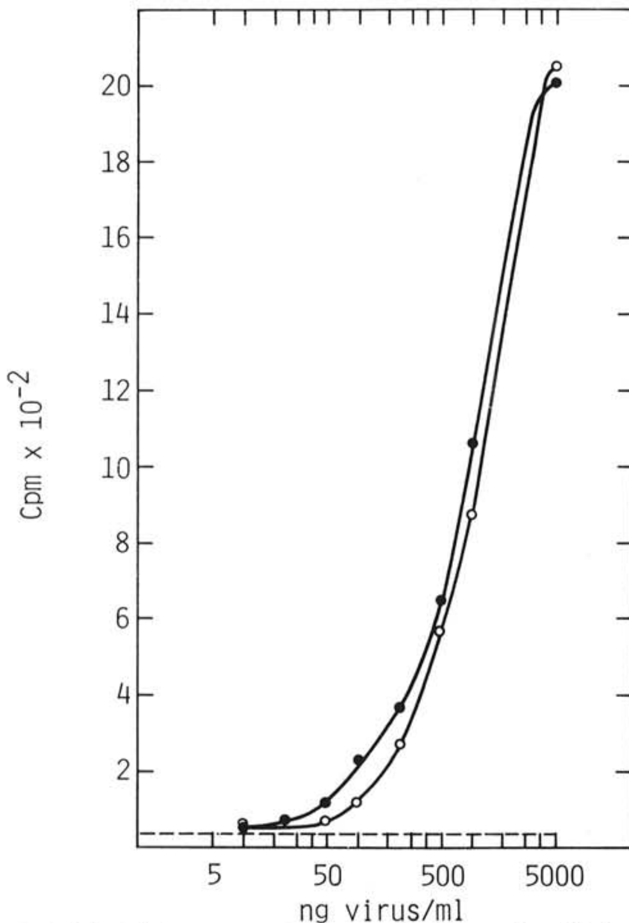

Fig. 3. Relationship between cpm (count per minute) bound in solid-phase radioimmunoassay and concentration of purified soybean mosaic virus diluted in 0.05 M sodium borate (pH 7.2) containing 0.5% $(NaPO_3)_{13}$ (●——●) or in extracts of healthy seed prepared in 0.05 M sodium borate, pH 7.2 (o——o). Values of buffer and healthy seed extract were negligible (dotted line).

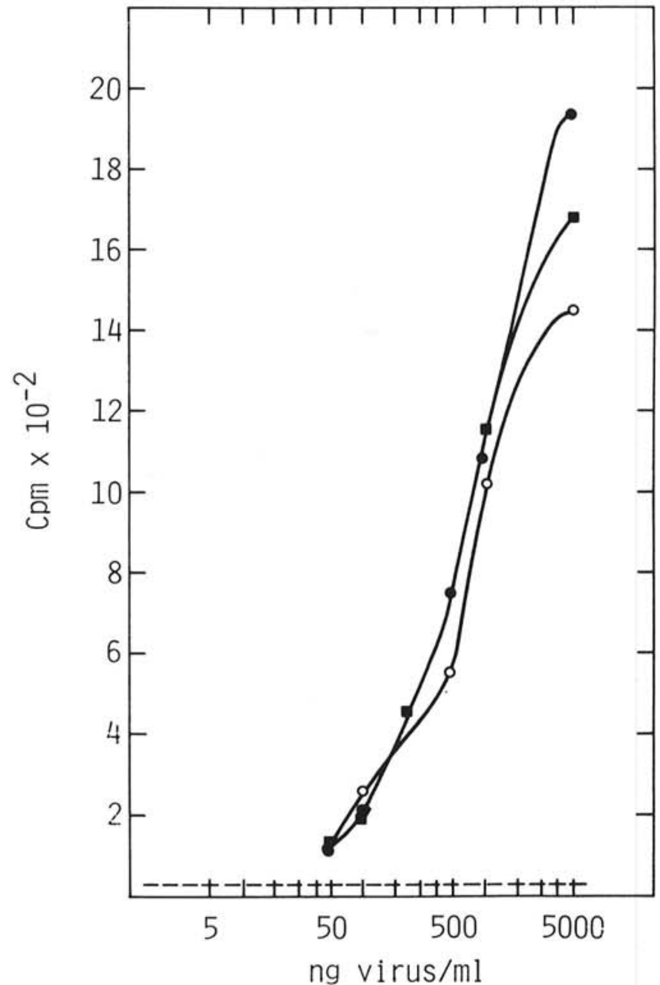

Fig. 4. Comparison of binding curves obtained with soybean mosaic virus (SMV) isolate Ia 75-16-1 (■——■), and isolates from strains G-1 (●——●), and G-2 (o——o). IgG used for coating and labeling was isolated from anti-SMV Ia 75-16-1 antiserum. Cpm (counts per minute) bound in the presence of maize dwarf mosaic virus, strain A, cowpea mosaic virus, southern bean mosaic virus, and tobacco mosaic virus were negligible and comparable to background buffer (dotted lined).

1,000 ng/ml. Binding curves relating virus concentration and bound activity were similar for purified virus in buffer and virus added to healthy seed extracts (Fig. 3); therefore, valid comparisons of virus concentration could be made between standard virus preparations and virus in extracts.

When SMV isolates from strains G-1 and G-2 were substituted for SMV 75-16-1, binding curves comparable to those obtained with the homologous system were observed (Fig. 4). Similar results were obtained with isolates from strains G-3 through G-7 (*unpublished*). Purified MDMV-A, another member of the potyvirus group, failed to evoke an increase in cpm bound above background levels, even at concentrations as high as 5,000 ng/ml. Similar results were obtained with purified SBMV, CPMV, and TMV.

Assay specificity was confirmed by using a neutralization or blocking test. The reaction of bound virus with ^{3}H-anti-SMV-IgG could be reduced by 50–90% by incubating the beads with unlabeled anti-SMV-IgG before addition of the labeled antibody. Equivalent concentrations of normal rabbit IgG had no effect.

Reproducibility of SPRIA. Variations in performance of the assay, evaluated by comparing test results of 13 assays obtained with purified virus over a 3-mo period, showed variations in cpm bound at virus concentrations ranging from 50 to 5,000 ng/ml were consistently within the 95% confidence limits. To determine intra-assay reproducibility, five parallel determinations were run on the same sample. Again, the experimental values fell within 95% confidence limits.

Virus content of seeds. Experiments evaluating the antigen content of 94 healthy half-seeds, shown to be antigen-free by testing the opposite half-seed with SPRIA, revealed a mean level of 20 cpm above background ambient for a healthy half-seed. The virus antigen content of 59 half-seeds, shown to contain antigen by testing the opposite half-seed with SPRIA, was determined, after subtracting the background contribution of the seed (20 cpm), by linear interpolation from a standard curve established concurrently with each assay (Fig. 3). A frequency distribution plot, relating ng SMV per half-seed to the number of half-seeds containing that amount, revealed considerable variation in virus content (Fig. 5). From these data, the mean virus antigen content of a single seed was calculated to be 474 ng. By using the virus particle molecular weight of 6.13×10^7 (14), we calculated that 474 ng was equivalent to 4.64×10^9 virus particles in a single seed, assuming that all virus antigen were present as particles. Although this value may vary for different cultivars, previous investigations using ELISA suggest virus concentration in seeds of various cultivars may be similar (24).

Sensitivity of SPRIA for detecting SMV in seeds. In a duplicated sensitivity experiment, artificially prepared samples (made by mixing infected seed halves shown to contain antigen by testing the opposite half-seed with SPRIA and uninfected seed) were analyzed to evaluate the sensitivity of SPRIA for detecting SMV in batches consisting of 100 seeds. Samples containing 0, 1, 5, and 10% of seeds containing SMV antigen yielded mean readings of 45, 53, 105, and 141 cpm, respectively. In these mixtures, a 5% level of seeds containing virus antigen, representing ~2,100 ng of total SMV antigen in the samples, could be distinguished from background levels.

Comparison of SPRIA with symptom expression for detection of SMV. Seeds harvested from plants of the cultivar Corsoy mechanically inoculated with SMV were planted, and 75 seedlings were examined for SMV symptom expression. Extracts for SPRIA were made from disks punched from single leaves. Of 30 seedlings judged by visual inspection to be infected, 27 reacted positively in the serological tests. Three of 20 seedlings with questionable symptoms gave a positive SPRIA reaction, and 3 of 25 seedlings, which showed no apparent visual symptoms, gave positive SPRIA reactions.

Effect of infection levels on yield and antigen content of seeds. Yield reduction of soybean plants inoculated with various levels of SMV was positively correlated ($r = 0.81$, $P = 0.01$) with increasing percentage of inoculated plants (Table 2). The regression equation relating percentage yield loss (Y) to percentage plants inoculated (X) was $Y = 0.34X + 5.22$.

The SMV antigen content of 100-seed lots from these plants was correlated in a similar manner ($r = 0.90$, $P = 0.01$) with percentage of inoculated plants (Table 3). The regression equation $Z = 0.44X + 2.29$ relating percentage of plants inoculated (X) to percentage of seeds containing virus antigen (Z) was calculated from these data. The low amount of virus antigen detected in the treatment containing uninoculated plants may have been caused by experimental error or by inadvertent contamination.

Relation between presence of antigen in seed and seed transmission of SMV. Groups of 100 seeds, whose virus antigen content was determined by SPRIA, were planted in the greenhouse. Progeny seedling were indexed for SMV by Ouchterlony double-diffusion tests (17). The ratio between percentage of seed with infected progeny seedlings and percentage of seed containing virus antigen varied most frequently between 0.05 and 0.12 (Table 3). These data suggest that, when 100% of the seeds in a seed lot contain virus antigen as determined by SPRIA, 5–12% of such seeds will produce infected progeny seedlings in the cultivar Amsoy 71.

Detection of SMV in aphids. Virus could be detected in aphids fed on SMV-infected leaves, but only if large numbers of aphids were used. Although an extract prepared from 65 aphids gave a higher SPRIA activity than controls, counts were relatively low (78 and 41 cpm, respectively). Larger samples were not tested.

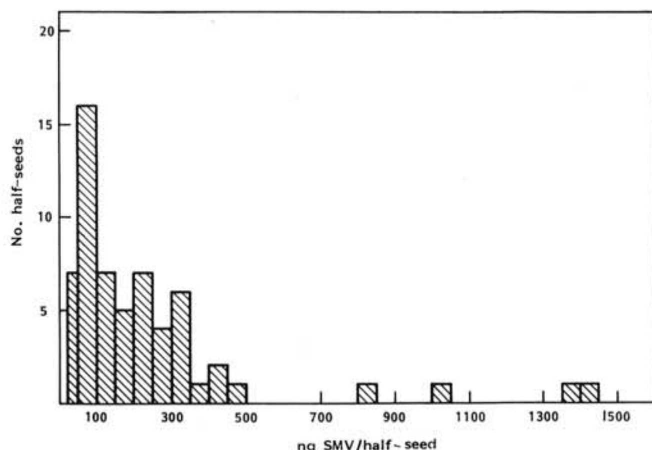

Fig. 5. Frequency distribution of soybean mosaic virus antigen content of individual cultivar Williams soybean seed halves as determined by solid-phase radioimmunoassay.

TABLE 2. Percentage yield reduction, virus antigen content, and percentage of seeds containing virus antigen from cultivar Amsoy 71 plants inoculated with various levels of soybean mosaic virus (SMV) in the greenhouse[a]

Inoculated (%)	Yield reduction (%)	SMV antigen per 100 seeds (ng)	Seeds containing SMV antigen[b] (%)
0	0.0	2,537	5.6
5	19.6	2,700	5.9
10	13.4	3,312	7.3
20	9.1	4,425	9.7
30	0.0	1,512	3.3
40	25.9	14,837	32.7
60	18.1	8,400	18.5
80	37.2	18,375	40.5
100	41.6	21,885	48.2
	0.66	0.81	0.81

[a] Data are mean values of eight replicates consisting of 80 plants each.
[b] Percentage = total nanograms of SMV antigen in 100 seeds divided by 474 ng SMV antigen per seed.

DISCUSSION

In spite of its proven diagnostic value with human and animal virus diseases, radioimmunoassay has received limited attention for plant virus assay and detection (1,20,27,30). Recently, however, new interest has been generated in radioimmunological procedures for plant virus detection (11,18). Although sensitivity of the double-sandwich ELISA may be high, its utility for quantitative assay of a potentially large range of naturally occurring strains may sometimes be limited (2,6,13,22,33,37,38). Indirect ELISA methods may alleviate some of these difficulties, but they require the use of anti-viral antibodies from two different animal species (23,39). In this study, homologous and heterologous SMV isolates reacted similarly. Thus, SPRIA possess broad cross reactivity for different SMV isolates in contrast to the results of Ghabrial and Shepherd for detection of cauliflower mosaic virus (11). In their system, ^{125}I was selected as the labeling nuclide.

Radioactive iodine (^{125}I or ^{131}I), often used for radioimmunoassays, can alter biological and physiochemical properties of proteins and give products with short half-lives. Tritiation is a gentle reaction which yields labeled reagents that can be stored for long periods without significant losses in specific activity (10,21,35). In addition, ^{3}H is a beta rather than a gamma emitter, and fewer safety precautions are needed during its use. Unfortunately, the specific activity that can be achieved with ^{3}H is low compared with that possible with ^{125}I or ^{131}I. A second disadvantage involves the greater instability of ^{3}H with respect to stable hydrogen atoms. During storage, tritiated compounds tend to lose their label through exchange with environmental hydrogen. Tritiation exerted little or no effect on immunoreactivity, immunospecificity, or physical properties of the IgG. Full immunoreactivity was retained for at least 1 yr even though nearly 58% of the radioactivity was lost (*unpublished*). These observations suggested that ^{3}H would be satisfactory for virus detection in a SPRIA system.

Spherical polystyrene beads were chosen as the solid phase because of their uniformity and large surface-to-volume ratio. Beads were easily sensitized with antibody by passive adsorption. Saturation of binding sites on polystyrene surfaces occurs in 3 hr or less. The binding of approximately 0.1 μg of protein to each 3.4-mm-diameter bead compares favorably with reports (12,40) that 6.4-mm-diameter beads adsorb approximately 1 μg of protein. Saturation of binding sites on polystyrene surfaces occurs within 3 hr.

The antibody-coated beads in this study were air-dried after adsorption and stored at 4 C. Drying and storage for at least 3 mo had no apparent effect on binding capacity of the beads. These factors would be important to the development of diagnostic kits for plant virus detection by SPRIA.

An optimal level of labeled antibody is critical for detection of bound antigen. For many radioimmunoassays, an amount of labeled reagent equivalent to a specific radioactivity content is arbitrarily chosen. Hutchinson and Ziegler (19) demonstrated the fallacy of this approach and the more reliable use of an optimum labeled protein concentration for providing maximum binding ratios and maximum assay sensitivity. For the SMV assay, maximum binding ratios were obtained at protein concentrations of 1–5 μg/ml.

Binding activity as a function of incubation conditions with ^{3}H-IgG was similar to that seen with antigen incubation except that clearly defined saturating levels were reached when antigenic sites became filled with labeled antibody. Virus was detected after 30 min of incubation with ^{3}H-IgG, but binding activity increased significantly with longer incubation periods. The effect of temperature was pronounced; binding was reduced at low temperatures.

Aggregation of virus particles, a phenomenon common to members of the potyvirus groups, caused difficulty in obtaining a stoichiometric relationship between virus concentration and bound activity. This was alleviated by diluting purified virus with BB containing 0.5% $(NaPO_3)_{13}$ or healthy seed extract prepared in BB. Extraction and dilution of infected seeds in BB resulted in binding curves similar to those obtained with purified virus. Extraction of leaf tissue in BB also resulted in virus detection by SPRIA in contrast to results obtained by Hill et al (15) when ELISA was used as the test system.

Purified SMV was detectable at levels of 25 ng/ml. When virus content of a sample is high, the amount of antibody on the bead surface becomes limiting and the binding curve plateaus. Leveling out of the curve at low antigen concentrations reflects attachment of labeled antibody to very sparsely distributed antigen on the bead surface.

Because SPRIA is a serological test, it detects viral antigen that includes both infective and noninfective virus. Tests made to compare symptom expression in progeny seedlings with SPRIA of the seedlings showed good agreement. Tests on 100-seed batches of infected cultivar Amsoy 71 infected seed suggested that an apparent 100% level of seed containing virus antigen corresponded to an actual 5–12% level of infected progeny seedlings. Such results are expected because SMV can reside in seed parts other than the embryo (4,29). The ratio between percentage of seed with infected progeny seedlings and percentage of seed containing antigen may vary with different cultivars, however.

An important criterion of a good indexing method is satisfied if laboratory-determined levels of virus in seeds correlate with field performance of the seeds. Preliminary experiments conducted in the greenhouse indicated that a significant correlation existed between percentage of plants inoculated with SMV, yield reduction, and antigen content of seeds from inoculated plants. It should be possible to estimate permissible levels of seedborne inoculum before yield losses induced by a pathogen become economically important. For SMV, predicted losses would initially exclude those caused by secondary aphid spread.

Analyses of seeds from field trials of 15 soybean cultivars demonstrated the utility of SPRIA for indexing field-grown seed (5). SPRIA can distinguish between slightly and severely infected samples and possesses the capacity for indiscriminate detection and quantification of heterologous virus strains. Thus, SPRIA is attractive for providing quantitative measures of specific agents in seed lots. Such test may provide seed producers with a method for establishing virus tolerance limits of seed lots intended for distribution and should become useful for the surveillance of viruses and possibly other seedborne pathogens.

TABLE 3. The relationship between soybean mosaic virus (SMV) antigen content in seeds determined by solid-phase radioimmunoassay and infected progeny seedlings indexed by Ouchterlony double-diffusion tests

Seed lot	Total SMV antigen/100 seeds (ng)	Seeds containing SMV antigen (A) (%)	Infected progeny seedlings (B) (%)	Ratio B/A
1	25,900	54.6[a]	4.1	0.07
2	30,800	65.0	7.6	0.12
3	28,900	61.0	3.1	0.05
4	15,600	32.9	8.6	0.26
5	31,000	65.4	5.1	0.08
6	13,500	28.5	2.1	0.07
7	44,900	94.7	5.1	0.05
8	44,400	93.7	6.4	0.07
9	9,100	19.2	10.0	0.52
10	41,300	87.1	10.3	0.12

[a] Percentage = total nanograms of SMV antigen in 100 seeds divided by 474 SMV antigen per seed.

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