

## Serological Detection of Seed-Borne Barley Stripe Mosaic Virus by a Simplified Radial-Diffusion Technique

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The authors are indebted to R. I. Hamilton for providing Leonil SA detergent, and for suggesting the use of sodium chloride for dissociating barley stripe mosaic virus.

Accepted for publication 26 March 1975.

### ABSTRACT

A simplified radial-diffusion test which eliminated tissue grinding, well cutting, and chemical treatment of the antigen prior to exposure to antibody was developed for the serological detection of barley stripe mosaic virus. Immunospecific reactions were assessed 24-36 hours after embedding leaf tissue directly into a 0.5% agar matrix containing antiserum and an appropriate virus dissociating reagent. Approximately 500-600 specimens could be assayed in a 90-mm petri plate at the rate of 100-200 samples per person per hour. This radial-diffusion test could detect about

1  $\mu\text{g}$  BSMV per ml, approximately a 10-fold greater sensitivity than conventional double-diffusion tests. Assay sensitivity was affected by the choice of dissociating agent and the aggregation state of BSMV injected into rabbits. In general, 0.5% Leonil SA detergent and antiserum against dissociated BSMV (D-protein) in the agar matrix gave the best results. It is proposed that BSMV D-protein antiserum should be used for immunodiffusion-in-gel serodiagnosis of seed-borne BSMV.

Phytopathology 65:948-955

*Additional key words:* mass testing, formalinized protein.

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Barley stripe mosaic virus (BSMV) is a seed-borne, rod-shaped virus without known vectors (3, 15, 20). Only barley, *Hordeum vulgare* L., and wheat, *Triticum aestivum* L., have been found naturally infected (20), and substantial yield reductions have been reported for both

of these economically important crops (5, 6, 15, 21). These facts indicate that eradication of BSMV would be desirable and that a virus-free seed program founded on a dependable, convenient assay for the virus in seed might help to achieve this objective.

A variety of approaches has been tried by others to detect and eliminate seed-borne BSMV. Screening seed lots grown under high light intensities was proposed to detect seed-borne infections (14). However, latent infections may be encountered (19, 22), physiological disorders may mimic virus-induced symptoms (11), symptom expression can vary with the environmental conditions (11, 18), and some virus strain-host combinations do not express marked symptoms. Unfortunately, there is no reliable local lesion host (20). Moreover, no way is known to free infected seed of the virus. For example, heat treatments (18, 35) and ultraviolet irradiation (10) failed to selectively inactivate BSMV in seed without corresponding damage to the embryo.

Some of these limitations and disadvantages in the detection of BSMV were avoided in a serological test devised by Scott (26) for detecting BSMV in single seeds. However, nonspecific reactions occurred and 2-3 weeks elapsed before an immunospecific response could be determined. Later, Hamilton (11, 12) developed a useful double-diffusion system. Barley embryos were crushed onto filter paper disks which were placed on the surface of an agar gel near another paper disk impregnated with anti-BSMV serum. A detergent, Leonil SA, was incorporated into the agar to prevent virus aggregation and to dissociate the viral capsid into smaller, more rapidly migrating antigens. Although these tests were rapid and specific, their preparation was also somewhat laborious and time-consuming.

Innovations have been made by others with similar problems in the detection of rod-shaped viruses which do not diffuse well through an agar matrix. For example, Shepard (28) and Shepard and Secor (29) developed a radial-diffusion technique for a mass-testing program for potato viruses that was approximately 10-fold more sensitive than the more commonly used double-diffusion test. It was observed that protein of dissociated potato virus X (D-protein) elicited a different immunogenic response in animals than did undissociated virus (28, 31). Hence, the sensitivity of the test was dependent upon an antiserum of satisfactory quality prepared by injecting D-protein into serum-producing animals.

Based on the increased sensitivity observed for potato virus X (PVX), and the greater number of samples that could be handled in an individual plate with a radial rather than a double-diffusion test, we decided to investigate the feasibility of testing barley for BSMV by a similar technique. We were interested in developing a process applicable to screening a large number of samples with a minimum investment of time and effort. A simplified radial-diffusion test is described herein along with information on its reliability and the relative sensitivity of agar-gel systems for BSMV detection, as well as the comparative effect of various agents for dissociating virus protein. A preliminary description of the test has been published (33).

**MATERIALS AND METHODS.**—*Source of virus isolate.*—The BSMV isolate used in these studies was collected from a naturally infected barley plant near Tulelake, California. A single leaf with distinct mosaic symptoms was triturated in a few drops of distilled water and the homogenate rubbed onto barley, *Hordeum vulgare* L. 'CM 67'. Seed collected from one of the

inoculated plants was germinated and the infected progeny were used as the initial inoculum source to propagate the virus for purification and serological purposes. The isolate produced a distinct mosaic on a number of barley and wheat cultivars and chlorotic local lesions on *Chenopodium amaranticolor* Coste & Reyn. Anti-BSMV serum provided by T. W. Carroll, Montana State University, was used to confirm the identity of the virus.

*Virus purification.*—The procedure for virus purification was similar to that of Lane (17). Tissue was collected 11-22 days after mechanical inoculation and, after being cut into small pieces with scissors, it was blended fresh at 4 C in two volumes of 0.1 M sodium phosphate (pH 7.6) containing 0.003 M disodium ethylenediaminetetraacetate (EDTA). Chloroform was added during blending at the rate of 25 ml per 100 g tissue. The aqueous phase was collected after centrifugation at 5,000 g for 10 minutes. BSMV was precipitated from this supernatant by adding solid polyethylene glycol 6,000 (PEG) to 6% (w/v) followed by 1/20th volume of 5 M NaCl with stirring at 4 C for 1-2 hours. The precipitate was collected by centrifugation for 15 minutes at 10,000 g in an angle rotor and the pellets were resuspended overnight in about one-tenth the volume of 0.01 M Tris-HCl (pH 7.2) containing 0.001 M EDTA. The extract was precipitated with PEG and NaCl a second time to reduce further the volume when large quantities of tissue were being handled. Purification was completed by two cycles of differential centrifugation. Pellets were resuspended in 0.01 M Tris-HCl (pH 7.2) containing 0.001 M EDTA, and the final preparation was stored at 4 C. It was found that resuspension of discarded insoluble material at each stage of purification (excluding clarification) in 0.01 M Tris-HCl (pH 7.2) containing 0.001 M EDTA and 0.1% Triton X-100 resulted in the recovery of an additional 3-20% BSMV. Concentration was determined spectrophotometrically using an extinction coefficient of 2.7/mg/ml at 270 nm when the virus was suspended in 0.1 M sodium phosphate (pH 7.0) and 0.1% sodium dodecyl sulfate (SDS) (17). Yields were approximately 1.5-13 mg BSMV per 100 g tissue, but varied with factors such as the time of year and the number of days the tissue had been infected before it was harvested.

*Preparation of BSMV and its D-protein as antigens.*—BSMV stored at 4 C was injected into rabbits without stabilization, or was stabilized prior to injection by dialysis against 0.2% formaldehyde in 0.005 M borate (pH 9) for 24 hours. Excess formaldehyde was removed by dialysis against borate buffer (7).

BSMV was prepared for dissociation by dialysis against 0.005 M borate (pH 8.2). An equal volume of freshly prepared 5% pyrrolidine was then added to the preparation (28). The resulting D-protein was stabilized by dialysis for 24 hours against 0.37% formaldehyde in 0.05 M borate (pH 8.2), and the unreacted formaldehyde was removed by dialysis against 0.005 M borate (pH 8.2).

*Antiserum production.*—Rabbits injected with virus (BSMV) or formalinized virus (F-BSMV) were given an initial immunization consisting of 4 mg virus emulsified 1:1 (v/v) with Freund's incomplete adjuvant injected subcutaneously between the shoulder blades, and 2 mg given intravenously in the marginal ear vein. One week later, another subcutaneous injection of 4 mg of

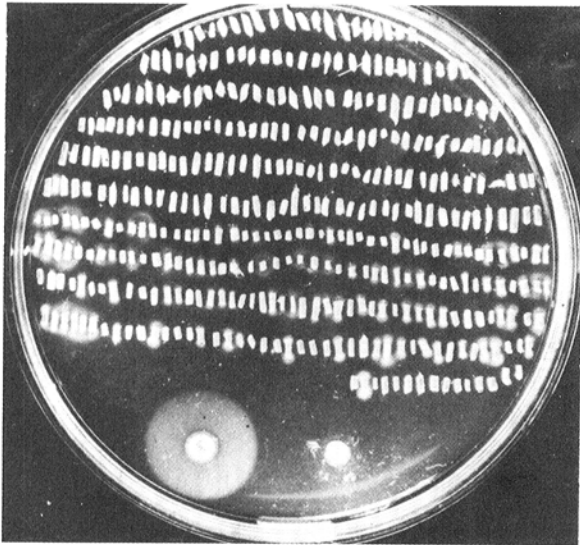


Fig. 1. A 90-mm simplified radial-diffusion plate containing 400 barley samples. The top 200 samples were from a virus-free seed lot, and the bottom 200 samples from a BSMV-infected seed lot. The two wells were filled with BSMV-infected barley sap (left) and healthy barley sap (right). BSMV D-protein antiserum at a 1:20 (v/v) dilution was incorporated into 0.5% Ionagar No. 2 buffered with 0.05 M Tris (pH 7.2) containing 0.85% NaCl and 0.5% Leonil SA detergent. Positive reactions were scored by the presence of opaque precipitates.

TABLE 1. Results of three comparative assays for seed-borne barley stripe mosaic virus in germinated barley

Experiment	Samples tested	Number of positive reactions		
		Symptoms <sup>a</sup>	Serology <sup>b</sup>	Local lesions <sup>c</sup>
1	19	10	10	10
2	36	14	13	12
3	48	12	10	9
Total	103	36	33	31

<sup>a</sup>Symptoms were recorded approximately 2 weeks after germination.

<sup>b</sup>The simplified radial-diffusion test was used.

<sup>c</sup>Two leaves of *Chenopodium amaranticolor* were used per sample.

TABLE 2. Serological assay for seed-borne barley stripe mosaic virus in commercial seed lots by the simplified radial-diffusion technique

Barley cultivar <sup>a</sup>	Seed lots tested (percent infected) <sup>b</sup>				
	1	2	3	4	5
Conquest	2				
Firlbecks III	48.5	12			
Klages	4	2.5	1.5	1	0
Larker	0	0			
Traill	7				
Unitan	8				
Wocus	2.5				
Wocus 71	3	0			

<sup>a</sup>Seed, designated for sale to growers in 1974, was obtained from commercial sources.

<sup>b</sup>Assays consisted of 200 samples per seed lot.

emulsified virus was administered. After 3 weeks, a final subcutaneous dose of 2 mg was injected.

Antibodies to D-protein were stimulated by administering four intramuscular injections, 1.5 mg each, in the thigh muscle at 1-week intervals. D-protein was emulsified 1:1 with Freund's incomplete adjuvant. After 3 more weeks, another 0.5 mg of D-protein without adjuvant was injected intravenously into the marginal ear vein.

*Serological tests.*—Serum titers were determined by the ring test. The assay consisted of overlaying approximately 0.1 ml of 0.1 mg BSMV per ml onto 0.4 ml of twofold dilutions of BSMV antiserum made with 0.85% NaCl which contained 10% glycerol. The presence or absence of precipitates was determined at intervals over a 2-hour period.

The double-diffusion technique developed by Hamilton (11, 12) and the radial-diffusion method of Shepard (28) were compared. Chemical additives were exchanged in modifications of these techniques as needed.

**RESULTS.**—In preliminary tests the agar double-diffusion technique of Hamilton (11) was used to assay for seed-borne BSMV. In this technique, the embryo is utilized as the source tissue for serological tests. The antigen dissociates during migration toward antiserum against intact virus. However, as discussed later, when radial-diffusion tests were carried out by incorporating virus antiserum directly into the agar matrix, a nonspecific precipitate developed around antigen wells when embryos were used. This phenomenon prevented the use of the more sensitive radial-diffusion test for BSMV detection with excised embryos.

After the preparation of antiserum to the D-protein of BSMV, various modifications of radial-diffusion tests were examined using an assortment of reagents for dissociating the virus, and different tissue sources of BSMV. It was found that the primary leaf of germinated seedlings was a suitable tissue source for BSMV, and as convenient to prepare as extracted embryos when gel-diffusion tests were utilized to screen barley seed lots.

The technique was simplified further when tests showed that excised leaves from infected plants could be embedded directly in the agar matrix in such a way that virus antigens diffused from the cut ends of the tissue into the gel matrix and reacted with antibody forthwith to produce specific precipitates. It was possible to incorporate an appropriate detergent, or other reagent, for dissociating virus directly into the agar with the D-protein antiserum.

Experimentation showed that tissue could be inserted into a gel of Ionagar No. 2, of carefully prepared consistency, without breaking the continuity of the matrix. These agar gels (0.5%) were of a very soft, but somewhat elastic, consistency. More concentrated gels (1%) split away from the cut ends of the tissue during insertion.

The final sero-detection technique which was evolved for BSMV screening of barley seed lots was the following radial-diffusion test. Agar gel plates for the test were prepared by mixing appropriately diluted D-protein antiserum containing 1% sodium dibutyl-naphthalene-sulfonate (Leonil SA detergent, General Aniline and Film Corp., New York, N.Y.) and 0.4% sodium azide with an

TABLE 3. Relative sensitivity of radial- and double-diffusion tests for the detection of barley stripe mosaic virus dissociated by various chemicals when antiserum against dissociated virus, whole virus, or formalinized whole virus was employed

Antigen	Treatment <sup>d</sup>	Concentrations of antigens ( $\mu\text{g/ml}$ ) required for positive test <sup>a</sup>					
		Radial-Diffusion with three antisera <sup>b</sup>			Double-Diffusion with three antisera <sup>c</sup>		
		D-protein <sup>e</sup>	BSMV	F-BSMV <sup>f</sup>	D-protein <sup>e</sup>	BSMV	F-BSMV <sup>f</sup>
D-protein	None	1	...	...	10	100	100
	Leonil SA	1	...	...	10	...	...
	NaCl	$\pm 1$	...	$\pm 100$	10	...	1,000
BSMV	Pyrrolidine	$\pm 1$	$\pm 1$	1	10	10	10
	Leonil SA	1	$\pm 10-100$	$\pm 10-100$	1 <sup>g</sup>	1,000	1,000
	NaCl	$\pm 10-100$	$\pm 100$	$\pm 100$	10	10	10

<sup>a</sup>Results determined after 24 hours. Minus sign indicates no reaction detected.

<sup>b</sup>Antisera used at 1:10 (v/v) dilution. Antigens tested at 10-fold dilutions from 100  $\mu\text{g/ml}$  to 0.1  $\mu\text{g/ml}$ .

<sup>c</sup>Antisera used undiluted. Antigens tested at 10-fold dilutions from 1,000  $\mu\text{g/ml}$  to 0.1  $\mu\text{g/ml}$ .

<sup>d</sup>BSMV preincubated with appropriate dissociating agent 5-10 minutes before wells filled. Pyrrolidine (2.5%) not incorporated into agar, but Leonil SA (0.5%) and NaCl (8.5%) were incorporated into agar at the same percent they were incubated with antigen.

<sup>e</sup>Antiserum against BSMV dissociated with 2.5% pyrrolidine and stabilized by dialysis against 0.37% formaldehyde (D-protein).

<sup>f</sup>Antiserum against BSMV stabilized by dialysis against 0.2% formaldehyde.

<sup>g</sup>If BSMV was not preincubated, only 10  $\mu\text{g/ml}$  of antigen was detected.

equal volume of 1% liquid Ionagar No. 2 buffered with 0.1 M Tris-HCl (pH 7.2) containing 1.7% NaCl. The solutions were equilibrated in a 50 C water bath prior to mixing. A 15-ml aliquot of this mixture was poured into a 90-mm diameter plastic petri plate and allowed to solidify in a moist chamber at room temperature. After barley seed were germinated in a chamber under continuous mist for 5 days at 20 C, a 1-mm segment of the primary leaf was excised from each seedling where it protruded through the coleoptile. This leaf segment was embedded directly into the solidified agar matrix by grasping the tissue gently with forceps and forcing it through the surface of the gel. The petri plates were then incubated in a moist chamber for 24-36 hours to allow the precipitin reaction to develop. The reactions were evaluated by observation with a binocular microscope at 10- to 20-fold magnification while the plate was illuminated from the bottom with a dark field light box. Figure 1 shows the sort of reactions which develop in these plates when BSMV antigens diffuse from the leaf tissue into the agar medium and react with D-protein antiserum.

In the mass testing of barley seed lots for BSMV, samples were normally spaced approximately 1-mm within rows and 2-mm between rows in 90-mm diameter petri plates. At these spacings, inhibition of precipitin reactions was never observed when D-protein antiserum was used. Tests have shown that this technique is applicable for testing wheat as well as barley.

With this test, it was possible to detect viral antigen without the use of detergent (0.5% Leonil SA) or high salt (8.5% NaCl) in the agar, but the number and intensity of reactions were usually reduced compared with those in similar tissue from the same seedlings embedded in agar containing one of these agents. Incorporation of 0.1% SDS into agar did not improve assay sensitivity. A comparison of gels containing 0.5% Leonil SA or 8.5% NaCl showed that both gave positive tests for the same plants (adjacent sections of the primary leaf were used) but the precipitin bands were more intense with Leonil SA. It is probable that some of the precipitin reactions

obtained in gels without additives were due to the presence of naturally occurring rapidly diffusing antigens. Presumably, the addition of dissociating agents prevented aggregation and/or converted larger protein aggregates into more rapidly diffusing antigens.

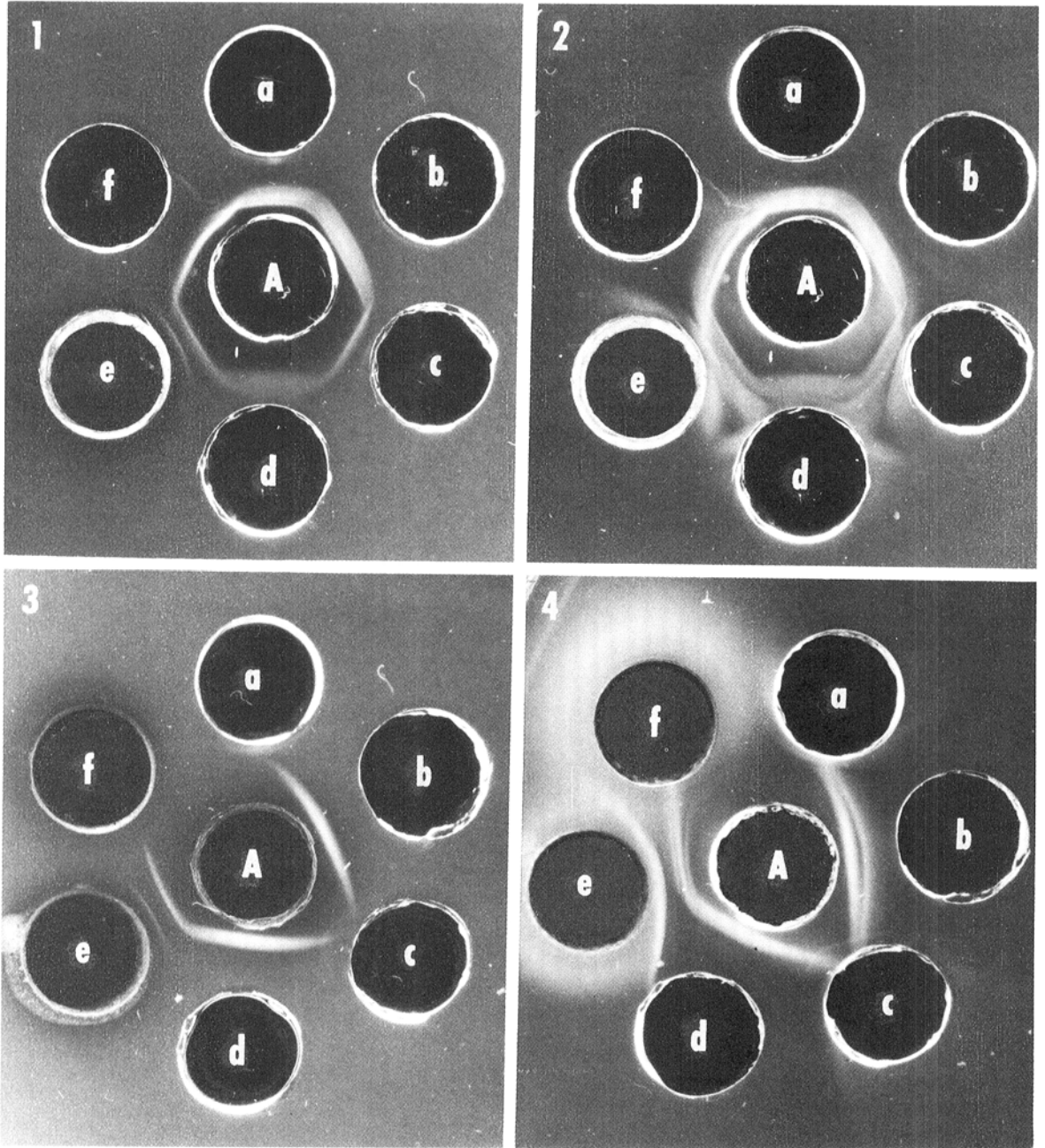
*Reliability of simplified radial test.*—Reliability of the simplified radial test was determined by testing for BSMV in individual seedlings by sero-assay, symptom expression, and local lesion formation on *C. amaranticolor* (Table 1) to see if the various tests confirmed one another. 'CM 67' barley was germinated under a continuous mist and a 1-mm section of the primary leaf excised as it emerged from the coleoptile for serological testing. The remainder of the seedling was placed on a screen above Hoagland's solution in an open container for further growth. After symptoms developed and were recorded (7-10 days), leaves were collected, triturated, and mechanical inoculations made on *C. amaranticolor*. The presence or absence of local lesions was evaluated 7-10 days later. There were no positive reactions by serology or local lesion testing without a corresponding positive test having been recorded for symptom expression. Hence, the serological test appears to be reliable and to be specific for virus-related protein.

*Survey of California barley seed lots.*—When this radial-diffusion test was utilized to survey registered or foundation seed of five fall-sown barley cultivars obtained from the California Crop Improvement Association, none of the nine seed lots tested was found to be infected. However, commercial seed lots of spring barley cultivars (Table 2), obtained from an area where BSMV was known to be a problem in the spring-sown crop, were carrying BSMV. These seed were not from a seed certification program. Another study (34) suggested that BSMV distribution within barley cultivars in California may be determined in part by the difference in the winter and summer growing seasons.

*Nonspecific reactions in radial tests.*—Hamilton (11) used extracted embryos to detect seed-borne BSMV which has the advantage that seed does not need to be

germinated. However, in radial-diffusion tests, nonspecific precipitation occurred around antigen wells when embryos were tested. Using virus-free seed, various treatments with crushed or uncrushed embryos in 5% pyrrolidine or crushed embryos in 0.01 M Tris (pH 7.2) all produced spurious precipitin reactions. Nonspecific

reactions also occurred around wells cut in 1% Bacto-agar and 1% Ionagar No. 2 which did not contain serum. A similar reaction was noted by Scott (26) who used triturated seeds in the Preer double-diffusion technique. In contrast, excised leaf tissue from virus-free seed ground in the presence of an equal amount (w/v) of 5%



**Fig. 2.** Double-diffusion test demonstrating the formation of precipitin bands with or without dissociating agents. Center wells (A) contained undiluted antiserum to BSMV D-protein. Antigen wells in 1 and 2 contained (a) BSMV at 1 mg/ml plus 0.5% Leonil SA detergent, (b) BSMV at 1 mg/ml plus 2.5% pyrrolidine, (c) BSMV at 1 mg/ml plus 8.5% NaCl, (d) formalized BSMV at 1 mg/ml plus 0.5% Leonil SA detergent, (e) BSMV-infected barley sap plus 0.5% Leonil SA detergent, and (f) D-protein at 1 mg/ml. Antigen wells in 3 and 4 contained (a) BSMV at 1 mg/ml, (b) D-protein at 1 mg/ml, (c) formalized BSMV at 1 mg/ml (d) D-protein at 1 mg/ml, (e) undiluted BSMV-infected barley sap, and (f) undiluted healthy barley sap. Gels were 1% Ionagar No. 2 buffered with 0.05 M Tris (pH 7.2) containing 0.85% NaCl. Photographs were taken 4 hours (1 and 3) and 24 hours (2 and 4) after the wells were filled.

pyrrolidine and introduced into wells cut into radial-diffusion plates produced no visible precipitate. No reactions were observed when excised primary leaves from germinated virus-free seed were embedded directly into agar containing D-protein antiserum and 0.5% Leonil SA or 8.5% NaCl. However, when 1% SDS was used, nonspecific precipitation was also encountered. SDS at 0.1% did not produce spurious reactions.

*Comparison of tissue-antigen source for immunodiffusion tests.*—Since Inouye (16) reported that electron microscopy revealed a higher percentage of embryos which appeared to contain BSMV particles than the percentage of plants which showed symptoms when germinated, the double-diffusion test of Hamilton (11) and the radial-diffusion test were used to evaluate tissue sources for sero-diagnosis. Thirty-three barley seeds were germinated, and after 3 days the seedlings were each dissected into three parts. One-half of the primary leaf and coleoptile and the remaining embryonic tissue were tested by double-diffusion. The remainder of the primary leaf and coleoptile was ground in the presence of an equal volume (w/v) of 5% pyrrolidine and tested by radial-diffusion. BSMV antiserum was used for the double-diffusion test and D-protein antiserum for the radial test. The serological tests showed the same 18 plants to be infected regardless of the test used or the tissue source, but five embryos also tested positive when the corresponding leaf tissue tested negative. This result indicated that embryos may contain BSMV-related protein without the developing seedlings becoming infected.

*Sensitivity of immunodiffusion tests.*—Tests were conducted to compare the sensitivity of the radial- and double-diffusion techniques for detection of BSMV protein. Table 3 shows the relative sensitivity of the two immunodiffusion tests using antiserum produced against different aggregation states of BSMV. Both D-protein and purified BSMV were used as antigens to distinguish between the degree of dissociation and the effect on antigen-antibody interaction due to the presence of a particular additive. Antisera to D-protein, BSMV, and F-BSMV with homologous titers of 128, 2,048, and 2,048, respectively, were used undiluted in double-diffusion trials or diluted 1:10 (v/v) for radial tests. BSMV was preincubated for 5-10 minutes at room temperature with each dissociating agent before introduction into wells.

The relative sensitivity of the two gel systems (Table 3) was comparable to levels reported for PVX (29). When D-protein antiserum was employed, 1  $\mu\text{g}/\text{ml}$  of D-protein could be detected in the radial system and 10  $\mu\text{g}/\text{ml}$  in the double-diffusion system. The homologous D-protein antiserum was much more sensitive than the heterologous BSMV and F-BSMV antisera. Results obtained by incubating intact BSMV with various reagents suggested that the difference in sensitivity was related to the degree of virus depolymerization. Based on the premise that the sensitivity of D-protein antiserum would increase, and that BSMV and F-BSMV antisera would decrease with depolymerization, Leonil SA was judged a more effective agent than NaCl or pyrrolidine. Indeed, data presented later describe the formation of additional precipitin bands with purified BSMV treated with NaCl or pyrrolidine, but not with Leonil SA which also suggested a difference in their dissociating capabilities.

There was some suggestion that dissociating agents

may interfere with antigen-antibody complexing. For example, BSMV antiserum and F-BSMV antiserum were more sensitive without Leonil SA or NaCl in double-diffusion tests with D-protein as the antigen (Table 3). However, since the homologous combination was not visibly affected, it could be postulated that the antibodies against intact virus had less affinity for the dissociated antigen, or that the molecular conformation of previously dissociated antigen was altered further. Although the former possibility would be a form of interference, it appears that the use of a homologous antiserum would alleviate this problem. The latter situation would correspond to a change in specificity related to depolymerization rather than interference.

*The effect of various dissociating agents.*—Immunodiffusion tests (Fig. 2) supported earlier studies (8, 9, 13) which indicated that Leonil SA dissociated the BSMV virion into more rapidly diffusing antigens which corresponded to nonsedimentable virus-related antigens found naturally in crude sap from BSMV-infected barley. The tests also showed that 8.5% NaCl and 2.5% pyrrolidine also dissociated purified BSMV into rapidly diffusing antigens which gave visible reactions in double-diffusion plates in 3-4 hours.

It should be noted that BSMV treated with 8.5% NaCl (Fig. 2) was either incompletely converted to the rapidly diffusing form, or reaggregated when the salt concentration was reduced. When 8.5% NaCl was incorporated in the agar, all bands corresponded to the more rapidly diffusing antigen. Pyrrolidine-dissociated BSMV also developed an additional precipitin band. However, this band was straight and did not correspond to late-forming bands following treatment with 8.5% NaCl. Formalinized BSMV virions (F-BSMV) were shown to be partially dissociated by 0.5% Leonil SA, but other tests have shown F-BSMV to be unaffected by 8.5% NaCl. In this case, the precipitin band closest to the antigen well eventually fused with similarly located bands from wells containing BSMV-infected barley sap treated with 0.5% Leonil SA and purified BSMV treated with 8.5% NaCl. This band corresponded to whole virus and indicated undissociated antigens in each of these treatments. Just as formalinization stabilized whole virus, barley sap components appeared to stabilize BSMV when exposed to Leonil SA. The same results (Fig. 2) were obtained with both BSMV antiserum and F-BSMV antiserum, except that the intensity of the rapidly forming precipitin bands was weakened.

**DISCUSSION.**—Radial-diffusion tests were shown to be practical for the detection of BSMV from the primary leaf of germinated seeds, and a simplified technique was developed to facilitate mass testing programs. The advantage of the simplified radial-diffusion test over existing gel techniques is that tissue grinding, well cutting, and chemical treatment of the antigen prior to antibody exposure are not required. Embryos were found unsuitable for radial-diffusion assays, since nonspecific precipitation occurred around wells.

The radial test was approximately 10-fold more sensitive than the corresponding double-diffusion test, which is consistent with similar tests for potato virus X (29). Assay sensitivity appeared to be dependent upon the dissociating agent used in gel tests, and the state of BSMV aggregation used for immunizing rabbits. Leonil SA at

0.5% appeared to be a more effective dissociating agent than 8.5% NaCl or 2.5% pyrrolidine on purified virus. However, crude sap seemed to partially stabilize BSMV. In all tests, D-protein antiserum was at least as sensitive as F-BSMV or BSMV antiserum and, judging from the intensity of recorded reactions, more specific for the dissociated protein.

Antigenic disparity between intact rod-shaped viruses and depolymerized states of the protein has received considerable attention. Rappaport (24) reported that tobacco mosaic virus (TMV) A-protein was composed of small aggregates of protein subunits, and that the size of these aggregates could be varied by altering the temperature. Double-diffusion tests revealed different precipitin lines at different temperatures and a correlation between the size of aggregates and antigenic specificity was proposed. Atabekov et al. (1) indicated that the subunit monomer of BSMV was serologically related, but not identical, to BSMV and BSMV protein aggregates. The smallest reported stable aggregate had a sedimentation coefficient of 10S and was thought to be composed of 7-10 subunits (2). Hence, this aggregate is analogous to TMV A-protein. In contrast to TMV A-protein, however, all serological reactivity for BSMV could be removed by absorbing BSMV antiserum with 10S or larger protein aggregates indicating that these aggregates were antigenically identical to whole virus.

Shepard and Shalla (31) and Shalla and Shepard (27) studied antigenic heterogeneity with PVX and its chemically dissociated protein. Their electrophoretic studies indicated that formalinized D-protein was composed primarily of monomers and dimers. In gel tests, antisera showed little cross-reactivity with the heterologous antigen. It was concluded that PVX protein underwent a conformational change on depolymerization that resulted in the formation of new antigenic determinants. Tobacco etch virus (TEV) may be similar, in this respect, to PVX in view of the serological heterogeneity of intact virus and its D-protein. Protein preparations which showed small homogeneous patterns by analytical centrifugation were less reactive with TEV antiserum than were heterogeneous protein preparations composed of larger aggregates (23), and primary antisera (4-5 weeks) to both TEV and TEV D-protein displayed little or no cross-reactivity with the heterologous antigen preparation (30).

Rappaport and Zaitlin (25) have also reported that antiserum produced in rabbits against succinylated TMV protein reacted strongly with succinylated TMV protein, less well with aggregated TMV protein, and slightly or not at all with intact virus. Thus, succinylated TMV protein appeared to exhibit antigenic disparity similar to that described for PVX D-protein at the monomeric-dimeric level. Since the monomer of BSMV was only partially recognized by antibodies against intact BSMV (1), and this work has shown a greater sensitivity of homologous antiserum for BSMV D-protein, BSMV may also exhibit antigenic heterogeneity.

We propose that BSMV D-protein antiserum should be used for the sero-diagnosis of seed-borne BSMV when the antigen is to be assayed in the dissociated state. It is not known whether strains of BSMV exist whose D-protein would not react with antiserum against whole virus or D-protein of another strain. However, the D-

proteins of two serologically distinct strains of PVX were shown to retain their individuality and cross-reactivity when tested against the appropriate D-protein antiserum (32). Also, it has been reported that long-term (12 weeks) antisera against TEV D-protein and potato virus Y (PVY) D-protein were both cross-reactive with 14 different PVY group viruses (30). In addition, the number of positive reactions recorded with the simplified radial test (Table 2) from different barley cultivars would suggest that strain recognition would not be a problem, and investigators in Montana (4) have used antiserum against intact BSMV to detect BSMV dissociated with Leonil SA for a number of years without reporting problems of this nature.

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