Evaluation of Three ELISA Methods as Alternatives to ISEM for Detection of the Wheat Spindle Streak Mosaic Strain of Wheat Yellow Mosaic Virus

K. R. ZAGULA, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824; D. J. BARBARA, Institute of Horticultural Research, East Malling, Maidstone, Kent, ME19 6BJ, United Kingdom; D. W. FULBRIGHT, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824; and R. M. LISTER, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

ABSTRACT

Zagula, K. R., Barbara, D. J., Fulbright, D. W., and Lister, R. M. 1990. Evaluation of three ELISA methods as alternatives to ISEM for detection of the wheat spindle streak mosaic strain of wheat yellow mosaic virus. Plant Dis. 74:974-978.

Immunosorbent electron microscopy (ISEM) has been used successfully for detecting wheat spindle streak mosaic virus (WSSMV) in infected wheat roots and leaves, but the method is laborious and time-consuming and requires the use of highly specialized equipment. To find a faster and routine alternative to ISEM, we evaluated direct double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and indirect protein A ELISA (PAS-ELISA) in microtiter plates and direct double-antibody sandwich ELISA on nitrocellulose membranes (dot-ELISA). Although all three ELISA methods detected WSSMV in three types of extract from infected wheat leaves, the dilution end points for detection were at least eightfold greater in PAS- and dot-ELISA than in DAS-ELISA. The dilution end point for detecting WSSMV by ISEM was 16-fold less than that for DAS-ELISA and over 100-fold less than that for the other two ELISA methods. In a pilot study, PAS-ELISA readily detected WSSMV in symptomatic as well as asymptomatic leaves from field wheat. Despite somewhat high background reactions in PAS-ELISA, both dot- and PAS-ELISA were found to be sensitive techniques and should prove useful in confirming suspected WSSMV infections.

Wheat spindle streak mosaic (WSSM) is one of several virus diseases affecting winter wheat throughout the world (15). In the past, diagnosis has been based

primarily on symptomatology, despite the fact that symptoms of WSSM can be confused with those of barley yellow dwarf, wheat soilborne mosaic, and wheat streak mosaic virus diseases. The disease is caused by the soilborne wheat spindle streak mosaic virus (WSSMV), a potyviruslike member of the barley yellow mosaic virus group (provisional grouping) (12). Wheat spindle streak mosaic virus is now regarded as a distinct strain of wheat yellow mosaic virus (WYMV) (17,19). Because all of our work concerns this particular strain of WYMV, we will continue to refer to it as WSSMV.

Immunosorbent electron microscopy (ISEM) was shown to be a useful technique to confirm identification of WSSMV (8,10). However, this method proved laborious and time-consuming for screening cultivars and experimental lines for virus (18), and ready access to a transmission electron microscope was necessary. Several enzyme-linked immunosorbent assays (ELISAs), including dot-immunobinding assays, have been used successfully to detect viral antigens in plants (2–6,11,16). In order to develop a rapid, sensitive, more routine method for reliably detecting WSSMV, we evaluated direct double-antibody sandwich ELISA (DAS-ELISA) in microtiter plates, direct DAS-ELISA on nitrocellulose membranes (dot-ELISA), and indirect protein A DAS-ELISA in microtiter plates (PAS-ELISA) as alternatives to ISEM. The suitability of PAS-ELISA for detecting WSSMV in field samples was also examined. A preliminary report of this work has been published (7).

MATERIALS AND METHODS

Antiserum preparation. Virus was purified as described elsewhere (9) from the soft red winter wheat cultivar Red-

coat collected at the Purdue University Agronomy Farm. The antiserum was prepared in a New Zealand white rabbit by initial injection at several sites (intramuscular and subcutaneous) of purified WSSMV emulsified 1:2 (v/v) in Freund's complete adjuvant followed by two similar multiple-site injections of virus 1:1 (v/v) with Freund's incomplete adjuvant 10 and 24 days after the initial injection. Serum was obtained from six bleeds collected biweekly starting 4 wk after the first injection. Antiviral antibody titer of antisera was assessed using ISEM and PAS-ELISA.

Immunoglobulin and conjugate preparation. Immunoglobulins (Igs) were precipitated with 50% saturated ammonium sulfate and purified by passage through a column of Whatman DEAE-cellulose according to Clark and Adams (5). Fractions containing Igs were pooled, diluted to a concentration of 1 mg/ml, and stored at −20 C in 1-ml aliquots. Immunoglobulins were conjugated to alkaline phosphatase (AP) (Type VII-NT, Sigma Chemical Co., St. Louis, MO) using a one-step glutaraldehyde method (5). Conjugation of Igs and protein A (PA) (Sigma) to horseradish peroxidase (HRP) (Sigma) was by the periodate oxidation method described by Barbara and Clark (3).

Preparation of antigen. Because in some virus/host systems antigen preparations must be clarified or partially purified for good results in ELISA, three types of plant extract were analyzed by ELISA. For crude sap preparations, leaves with distinct symptoms or leaves from uninfected control plants were ground to a powder in liquid nitrogen with a mortar and pestle, then further triturated in 10 vol of 0.1 M potassium phosphate buffer, pH 7.0, (PB) containing 0.01 M sodium diethyldithiocarbamate and 0.1% (w/v) sodium sulfite. Clarified sap was prepared by adding chloroform to the homogenate at a final concentration of 20% (v/v), stirring for 3 hr at 4 C, centrifuging at 7,700 g for 15 min, and collecting the aqueous phase. Partially purified extracts were prepared by adding polyethylene glycol (PEG), M_r 8,000 (5%, w/v) and NaCl (0.25 M) to the aqueous phase and stirring for 1 hr at 4 C. Precipitates were collected by low-speed centrifugation (12,000 g for 20 min) and resuspended overnight in 0.1 vol PB. Insoluble material was removed by low-speed centrifugation. Purified virus for standards was prepared by subjecting the virus suspension to a second precipitation with PEG and NaCl, followed by sucrose cesium sulfate step gradient ultracentrifugation (9). An extinction coefficient of 2.4 (12) was used to calculate virus concentration.

DAS-ELISA. Immulon 1 (low-binding capacity) microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with IgG prepared against WSSMV at 2.5 µg/ml in 0.05 M carbonate coating buffer, pH 9.6, for 3–4 hr at 37 C. Plates were washed at this point and between all other steps by flooding three times with 0.15 M phosphate-buffered saline (pH 7.4) containing 0.05% (v/v) Tween 20 (PBS-T). To block nonspecific absorption of sap components, 1% (w/v) bovine serum albumin (BSA) (Sigma) in PBS-T was added for 30 min at 37 C. Antigen preparations diluted in PBS-T were added for 2 hr at 4 C, followed by AP- or HRP-Ig conjugate diluted 1:400 (v/v) in PBS-T for 12 hr at 4 C. Finally, freshly prepared substrate was added—1 mg p-nitrophenyl phosphate per milliliter of 0.1 M diethanolamine buffer, pH 9.6, for AP and 0.5 mg o-phenylenediamine per milliliter of 0.025 M sodium acetate, pH 5.5, with 0.06% (v/v) hydrogen peroxide for HRP. Reactions with AP were stopped within 1 hr with 3 M sodium hydroxide, and absorbances were measured at 405 nm in a model EL308 ELISA reader (Bio-Tek Instruments, Inc., Winooski, VT). Reactions in plates with HRP were stopped with 3 M sulfuric acid after 30 min to 1 hr in the dark, and absorbances were measured at 490 nm. Readings in which the mean of the infected sap absorbances exceeded two times the mean of the healthy sap absorbances were considered positive. Three wells were evaluated per treatment (sap dilution) in each of three experiments for infected and uninfected test samples.

Dot-ELISA. A modification of the method described by Banttari and Goodwin (2) was developed for the detection of WSSMV by dot-ELISA. Nitrocellulose membranes (NCM) (0.45 µm BA 85 filters, Schleicher and Schuell, Inc., Keene, NH) were briefly wetted with distilled water and then floated in a petri dish containing IgG prepared against WSSMV at 2.5 µg/ml in coating buffer for 2 hr at 20 C. Membranes were rinsed three times within 15 min with 0.01 M tris-HCl buffer, pH 7.4, containing 0.9% (w/v) NaCl and 0.05% (v/v) Tween 80 (TBS-T), then blocked with 3% (w/v) BSA in TBS-T for 2 hr at 20 C. Antigen preparations (250 µl) diluted in TBS-T were added to the coated and blocked NCM and held, without vacuum, in a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, Richmond, CA) for 2 hr at 4 C. After removal from the apparatus, the NCM was washed vigorously with distilled water and further washed for 15 min with TBS-T plus 1% (v/v) Triton X-100 to remove any plant debris from the dots. The NCM was floated in AP or HRP conjugate diluted 1:200 (v/v) in TBS-T for 12 hr at 4 C. After washing with TBS-T, the NCM was floated in the appropriate substrate. For AP, 6 mg of fast red TR salt (Sigma) per milliliter of 0.2 M tris-HCl buffer, pH 8.2, was filtered and mixed 1:1 with a 0.1% (v/v) dilution of naphthol AS-

MX phosphate (Sigma). For HRP, 2 mg of 4-chloro-1-naphthol (Sigma) per milliliter of methanol was mixed 1:5 with TBS-T, and 0.1% (v/v) hydrogen peroxide was added. Reactions were stopped within 1 hr by rinsing the NCM in distilled water. Filters were air-dried and stored wrapped in foil to prevent color fading. Reactions were differentiated visually for both conjugates; for AP conjugates, attempts were made to quantitate reactions using a Model T-85CD color transmission densitometer (ESECO, Cushing, OK). Because a reflectance densitometer was unavailable, the ability of dry, unused nitrocellulose filters to transmit light was first tested. Although blank filters transmitted light uniformly, baseline readings were high (averaging 1.5 OD units); hence, reactions were evaluated only on color differences relative to the white NCM.

PAS-ELISA. The method of Edwards and Cooper (6) was adapted for detecting WSSMV by PAS-ELISA. Microtiter plates were coated with 1 µg of PA per milliliter of coating buffer for 2 hr at 20 C. Plates were washed between all stages with PBS-T, as for DAS-ELISA. A 1:1,000 (v/v) dilution of whole (trapping) antiserum was added for 2 hr at 20 C. Antigen dilutions in PBS-T were added and incubated 2 hr at 4 C. The second (detecting) antiserum (1:1,000 [v/v] dilution) was added for 2 hr at 20 C, followed by a 1:2,000 (v/v) dilution of PA-HRP conjugate for 12 hr at 4 C. Plates were developed and absorbances measured as for DAS-ELISA.

ISEM. To compare the sensitivity of ISEM with the three ELISA methods, Parlodion-coated carbon-backed grids were coated with a 1:500 dilution of whole antiserum in 0.06 M sodium phosphate buffer, pH 7.0, for 2 hr at 20 C, rinsed, and floated on antigen preparations for 3–12 hr at 4 C. After rinsing, grids were negatively stained with 2% (w/v) ammonium molybdate, pH 7.0, and examined with a Philips 201 transmission electron microscope at ×20,000.

Field studies. The utility of PAS-ELISA for testing field samples for WSSM was assessed. PAS-ELISA was chosen because it is more sensitive than DAS-ELISA and more easily quantified than dot-ELISA. In early June 1986, leaf tissue was collected from soft white winter wheat (cv. Ionia) growing at the Michigan State University Botany and Plant Pathology Farm in plots planted early (13 September 1985) or late (21 October 1985) in the season. Late planting of winter wheat is a recommended practice for reducing WSSMV incidence (20). Leaves from plants at the six-leaf stage were separated according to their positions on the plants. Lower leaves from early-planted wheat had begun to senesce but still showed characteristic mosaic symptoms, whereas middle leaves

showed moderate symptoms and upper leaves showed mild symptoms. Lower leaves from late-planted wheat showed only moderate symptoms, middle leaves showed fewer symptoms, and upper leaves were symptomless.

Data analysis. For comparing detection abilities between DAS- and PAS-ELISA methods, test plant material was collected and prepared on three consecutive days and duplicated three times per plate, resulting in three replicates per sample. Means of daily absorbance readings for both infected (I) and uninfected (U) samples at each dilution were averaged over the 3 days and converted to I/U ratios. For direct comparison of sensitivities between the two microtiter plate-based methods, differences in I/U ratios within a dilution were tested using the Mann-Whitney U-test.

For field studies, sampling was performed as indicated above and absorbance means between early- and late-planted wheat were compared by means of Student's t test.

For ISEM, virus particles were counted from 30 300-mesh grid squares, representing 10 randomly chosen grid squares per each of three grids per sap dilution.

RESULTS
Detection of WSSMV in crude, clarified, and partially purified extracts.

The three ELISA methods were evaluated for sensitivity in detecting WSSMV in three types of extract. Virus was readily detected by all three methods and in all three types of extract (Table 1). At equivalent dilutions (grams fresh weight of leaf tissue per milliliter of extraction buffer), absorbance values were highest for partially purified preparations. The dilution end point for detecting WSSMV in crude extracts by DAS-ELISA (approximately 1:16,000) was similar to that obtained for the Japenese strain of WYMV, which is serologically related to WSSMV (approximately 1:25,000) (16). The dilution end points for detecting WSSMV in all three types of preparation were at least eight times greater in PAS- and dot-ELISA than in DAS-ELISA (Table 1). With purified virus standards, both PAS- and dot-ELISA were able to detect as little as 1 ng of virus.

In dot-ELISA, AP conjugate was used routinely, since dots faded when HRP was developed with 4-chloro-1-naphthol. Positive colored dots were readily distinguishable visually from negative reactions against the light-colored NCM (Table 2), as the addition of Triton X-100 to the washing buffer virtually eliminated absorption of plant material to dots, greatly reducing nonspecific reactions. Use of the transmission densitometer did not improve the sensitivity of dot-ELISA over visual evaluation.

The I/U absorbance ratios for PAS-ELISA were significantly different (P <0.05) from those for DAS-ELISA at 1:1,000 to 1:256,000 dilutions of extract. Differences in ratios between the two tests at the two lowest sap dilutions were not significant, apparently because of the relatively high background readings. Thus, PAS-ELISA was significantly better than DAS-ELISA in discriminating between uninfected and infected samples at most dilutions, in addition to having an eightfold more sensitive detection level for WSSMV.

All antisera showed some nonspecific activity. As in earlier work (7), this did not affect ISEM results but gave somewhat higher reactions with uninfected wheat in all three ELISA methods, particularly at low sap dilutions (Table 2). Of the two microtiter plate-based ELISA methods, healthy plant reactions were higher with DAS-ELISA, although there was always at least a twofold difference between means of uninfected samples against those of infected plants when diluted 1:10 to 1:16,000 in this assay. We were unable to reduce these nonspecific effects by cross-absorption with extracts of healthy wheat prior to use in ELISA. Similarly, diluting the detecting antibody (PAS-ELISA) or conjugate (DAS-ELISA) in sap from uninfected wheat (14) failed to reduce readings for extracts from uninfected plants but instead often greatly increased those of buffer controls.

All three ELISA methods worked well only when antigen preparations were made from fresh tissue. Differences in the two plate-based methods between uninfected and infected tissue samples that had been frozen at -20 C or -80 C were occasionally ≥twofold at low extract dilutions, but the high nonspecific reactions at these low dilutions made differentiation between uninfected and

Table 1. Relative sensitivities of double-antibody sandwich (DAS-) ELISA, protein A (PAS-) ELISA, and dot-ELISA for detecting wheat spindle streak mosaic virus (WSSMV) in crude, clarified, and partially purified wheat leaf sap preparations

Sap preparation	DAS-ELISA (A_{405nm})	PAS-ELISA (A_{490nm})	Dot-ELISA (densitometer)
Crude	1:16,000[a]	1:128,000	1:128,000
Clarified	1:32,000	1:256,000	1:256,000
Partially purified	1:32,000	1:512,000	1:512,000
Purified WSSMV[b]	28.60	1.05	1.05

[a] Antigen dilution end points for positive reactions. Dilutions are grams fresh weight of leaf tissue per milliliter of extraction buffer.
[b] Minimum amount detected, in nanograms.

Table 2. Detection of wheat spindle streak mosaic virus in dilutions of crude wheat leaf sap by double-antibody sandwich (DAS-) ELISA, protein A (PAS-) ELISA, and dot-ELISA and immunosorbent electron microscopy (ISEM)

Sap dilution	DAS-ELISA (A_{405nm})[a] (mean ± SE)			PAS-ELISA (A_{490nm})[a] (mean ± SE)			Dot-ELISA[b] (visual evaluation)	ISEM[c]
	I	U	I/U	I	U	I/U		
1:10	1.73 ± 0.01	0.85 ± 0.02	2.04	2.32 ± 0.03	1.12 ± 0.03	2.07	+	246
1:100	1.53 ± 0.02	0.74 ± 0.01	2.07	2.05 ± 0.04	0.98 ± 0.02	2.09	+	18
1:1,000	1.26 ± 0.01	0.62 ± 0.02	2.03	1.90 ± 0.03	0.88 ± 0.05	2.16	+	1*[d]
1:2,000	1.03 ± 0.03	0.48 ± 0.02	2.15	1.56 ± 0.05	0.61 ± 0.03	2.56	+	0
1:4,000	0.80 ± 0.03	0.38 ± 0.03	2.11	1.31 ± 0.01	0.47 ± 0.04	2.79	+	0
1:8,000	0.56 ± 0.02	0.27 ± 0.03	2.07	1.11 ± 0.03	0.33 ± 0.02	3.36	+	0
1:16,000	0.45 ± 0.01*	0.22 ± 0.02	2.05	0.82 ± 0.02	0.30 ± 0.03	2.73	+	0
1:32,000	0.29 ± 0.03	0.16 ± 0.02	1.81	0.57 ± 0.03	0.24 ± 0.01	2.37	+	0
1:64,000	0.20 ± 0.04	0.13 ± 0.03	1.54	0.40 ± 0.06	0.17 ± 0.03	2.35	+	0
1:128,000	0.14 ± 0.05	0.10 ± 0.01	1.40	0.32 ± 0.02*	0.13 ± 0.03	2.46	+*	0
1:256,000	0.08 ± 0.03	0.08 ± 0.02	1.00	0.22 ± 0.02	0.12 ± 0.01	1.83	−	0

[a] Mean absorbance values and standard errors of three wells for each dilution in each of three experiments. I = infected samples, U = uninfected samples, I/U = ratio of infected to uninfected samples.
[b] + = Positive, − = negative.
[c] Means of virus particle counts per grid square from 30 300-mesh grid squares at × 20,000 magnification.
[d] Asterisk denotes approximate dilution end point for WSSMV detection for each assay, taken as: 1) absorbance values ≥twofold absorbance values for uninfected samples (DAS- and PAS-ELISA), 2) visual evaluation of positive dot coloration relative to the white (negative) nitrocellulose membranes (dot-ELISA), and 3) ≥one particle per grid square (ISEM).

Table 3. Occurrence of wheat spindle streak mosaic virus in crude wheat leaf extracts from upper (u), middle (m), and lower (l) leaves from early- and late-planted Ionia wheat as detected by protein A ELISA

Extract dilutions[a]	Absorbance (A_{490nm})[b]						
	Early-planted (mean ± SE)			Late-planted (mean ± SE)			
	u	m	l	u	m	l	C[c]
1:4,000	0.87 ± 0.04	0.91 ± 0.07	0.80 ± 0.03	0.55 ± 0.09	0.60 ± 0.03	0.72 ± 0.08	0.23 ± 0.06
1:8,000	0.63 ± 0.05	0.65 ± 0.03	0.56 ± 0.06	0.33 ± 0.07	0.41 ± 0.01	0.50 ± 0.09	0.19 ± 0.06
1:16,000	0.42 ± 0.02	0.53 ± 0.08	0.44 ± 0.02	0.26 ± 0.02	0.32 ± 0.03	0.40 ± 0.03	0.12 ± 0.07
1:32,000	0.32 ± 0.02	0.41 ± 0.07	0.32 ± 0.03	0.20 ± 0.09	0.24 ± 0.05	0.20 ± 0.02	0.12 ± 0.03
1:64,000	0.25 ± 0.03	0.30 ± 0.06	0.24 ± 0.09	0.17 ± 0.03	0.18 ± 0.03	0.24 ± 0.06	0.10 ± 0.07
1:128,000	0.18 ± 0.06	0.25 ± 0.04	0.19 ± 0.08	0.14 ± 0.03	0.21 ± 0.08	0.19 ± 0.07	0.09 ± 0.08
1:256,000	0.16 ± 0.04	0.19 ± 0.08	0.15 ± 0.04	0.13 ± 0.05	0.14 ± 0.04	0.16 ± 0.09	0.08 ± 0.03

[a] Grams fresh weight of leaf tissue per milliliter of extraction buffer.
[b] Mean absorbance values and standard errors from three experiments, three wells per experiment.
[c] C = A composite of upper, middle, and lower leaves from uninfected wheat.

infected samples difficult. No antigen was detected by any ELISA method in tissue that had been ground in liquid nitrogen and stored frozen at −20 C or lyophilized.

Comparison with ISEM. The dilution end point for detecting WSSMV by ISEM (1:1,000) was 16-fold less than that for DAS-ELISA and over 100-fold less than that for the other two ELISA methods (Table 2). However, ISEM results may be affected by the lower volume used (20 μl in ISEM vs. 250 μl in ELISA), by grid square vs. plate well sizes, by clumping of virions, and by inhibitory surface charges on grids. In addition, only intact or partially broken rods are recognizable with ISEM, whereas highly fragmented or degraded particles as well as protein subunits may be detected with ELISA.

Field studies. PAS-ELISA readily detected WSSMV in all leaves from all samples (Table 3). In this pilot study, absorbance means of subsamples (i.e., means of absorbance for all leaf positions) for early-planted infected wheat were significantly different ($P < 0.01$) from those for late-sown infected wheat at 1:4,000 to 1:64,000 dilutions of extract using Student's t test. This indicates that either a greater incidence of infection or higher antigen levels occur in early-sown wheat; no attempt was made to differentiate these possibilities.

Virus was detected at a dilution of 1:16,000 in extracts of symptomless upper leaves from late-sown wheat (Table 3). ISEM also detected WSSMV in these leaves (and in leaves from all other samples), but only at high concentrations (≤1:100 dilution) of extract (data not shown). Two of us (KRZ and DWF) have observed for several growing seasons that although symptoms disappear when temperatures initially rise in the spring, WSSMV can still be isolated from these leaves. Symptoms usually reappear on these leaves after exposure to cool (10–15 C) temperatures for a few days

DISCUSSION

Although the detection levels of the three ELISA methods described here are comparable to those obtained with other similar viruses (2,11,16), they are surprisingly high compared with those obtained using ISEM. In some other host/virus systems, ISEM and ELISA are of comparable sensitivity (13). This may indicate that a considerable amount of fragmented particles and subunit antigen (not detected by ISEM) is present in the leaf extracts. WSSMV particles are fragile and break easily; grinding in liquid nitrogen was used because it reduces particle breakage (8,10). Therefore, excess subunit antigen, possibly arising as a result of tissue or virion degradation, may be responsible for the high detection levels.

A major problem in the detection of WSSMV by each ELISA method was the high nonspecific reactions from uninfected tissue extracts. It was initially assumed that these nonspecific reactions were due to antiplant antibodies, yet cross-absorption failed to reduce these reactions significantly. This suggests that they may arise, at least in part, from nonserological interactions between components of the assay; this phenomenon was noted when assays for hop latent virus were being developed (1). WSSMV has proved difficult to purify and we have been unable in several attempts to produce an antiserum with low background levels but with significant antivirus activity.

Despite the limitations imposed by the background reactions and the need to use only fresh samples, when used with care and adequate controls, DAS-, PAS-, and dot-ELISA were all useful techniques and were more sensitive than ISEM for detecting WSSMV in infected wheat. PAS-ELISA was more sensitive and less subject to high background readings than DAS-ELISA and had the added advantage of using unfractionated antiserum rather than purified IgG; it was also somewhat more time-consuming, however. Dot-ELISA was as sensitive as PAS-ELISA, and background reactions could be reduced by vigorous washing of filters. The availability of a reflectance densitometer with scanner for

quantification should make dot-ELISA a highly useful technique.

When using the PAS-ELISA reported here for evaluating wheat cultivars for WSSMV resistance, researchers at Cornell University have added 2% nonfat dry milk to the PBS diluent in the antiserum and conjugate incubations (N. R. Miller, S. M. Gray, and G. C. Bergstrom, *personal communication*). One of us (KRZ) recently found that use of this blocking agent resulted in increased (threefold to fivefold) differences between uninfected and WSSMV-infected fresh and frozen sample OD readings at low (1:10 to 1:200) antigen dilutions.

ISEM has been shown to be a useful tool for confirming a diagnosis of WSSMV (8) and is relatively unaffected by the somewhat poor quality of the currently available antisera, but it is labor-intensive and requires expensive facilities not always available. ELISA, especially PAS and dot, was found to be a rapid, sensitive, and relatively simple technique for detecting WSSMV. PAS-ELISA is currently being used to evaluate the incidence of WSSMV in field plots in Michigan and other states, and the method should prove useful to the wheat breeder when screening wheat cultivars and lines for virus resistance.

LITERATURE CITED
1. Adams, A. N., and Barbara, D. J. 1982. Host range, purification and some properties of two carlaviruses from hop (*Humulus lupulus*): Hop latent and American hop latent. Ann. Appl. Biol. 101:483-494.
2. Banttari, E. E., and Goodwin, P. H. 1985. Detection of potato viruses S, X, and Y by enzyme-linked immunosorbent assay on nitrocellulose membranes (dot-ELISA). Plant Dis. 69:202-205.
3. Barbara, D. J., and Clark, M. F. 1982. A simple

indirect ELISA using F(ab')₂ fragments of immunoglobulin. J. Gen. Virol. 58:315-322.

4. Berger, P. J., Thornbury, D. W., and Pirone, T. P. 1985. Detection of picogram quantities of potyviruses using a dot blot immunobinding assay. J. Virol. Methods 12:31-39.

5. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.

6. Edwards, M. L., and Cooper, J. I. 1985. Plant virus detection using a new form of indirect ELISA. J. Virol. Methods 11:309-319.

7. Haufler, K. Z., Barbara, D. J., Fulbright, D. W., and Lister, R. M. 1986. Detection of wheat spindle streak mosaic virus by ELISA and a dot-immunobinding assay. (Abstr.) Phytopathology 76:1132.

8. Haufler, K. Z., and Fulbright, D. W. 1983. Detection of wheat spindle streak mosaic virus by serologically specific electron microscopy. Plant Dis. 67:988-990.

9. Haufler, K. Z., and Fulbright, D. W. 1985. Purification and partial characterization of wheat spindle streak mosaic virus (WSSMV). (Abstr.) Phytopathology 75:1349-1350.

10. Haufler, K. Z., and Fulbright, D. W. 1986. Identification of winter wheat cultivars and experimental lines resistant to wheat spindle streak mosaic virus. Plant Dis 70:31-33.

11. Hibi, T., and Saito, Y. 1985. A dot immunobinding assay for the detection of tobacco mosaic virus in infected tissues. J. Gen. Virol. 66:1191-1194.

12. Hollings, M., and Brunt, A. A. 1981. Potyvirus group. No. 245 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.

13. Koenig, R., Lesemann, D.-E., and Burgermeister, W. 1984. Beet necrotic yellow vein virus: Purification, preparation of antisera and detection by means of ELISA, immunosorbent electron microscopy and electroblot immunoassay. Phytopathol. Z. 111:244-250.

14. Lister, R. M., and Rochow, W. F. 1979. Detection of barley yellow dwarf virus by enzyme-linked immunosorbent assay. Phytopathology 69:649-654.

15. Slykhuis, J. T. 1976. Wheat spindle streak mosaic virus. No. 167 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.

16. Usugi, T., Kuwabara, T., and Tsuchizaki, T. 1984. Serological detection of barley yellow mosaic virus, wheat yellow mosaic virus and soilborne wheat mosaic virus by ELISA. Ann. Phytopathol. Soc. Jpn. 50:63-68.

17. Usugi, T., and Saito, Y. 1979. Relationship between wheat yellow mosaic virus and wheat spindle streak mosaic virus. Ann. Phytopathol. Soc. Jpn. 45:397-400.

18. Van Koevering, M., Haufler, K. Z., Fulbright, D. W., Isleib, T. G., and Everson, E. H. 1987. Heritability of resistance in winter wheat to wheat spindle streak mosaic virus. Phytopathology 77:742-744.

19. Wiese, M. V. 1987. Wheat yellow mosaic (wheat spindle streak mosaic). Pages 83-85 in: Compendium of Wheat Diseases. 2nd ed. American Phytopathological Society, St. Paul, MN.

20. Wiese, M. V., Ravenscroft, A. V., and Everson, E. H. 1974. Incidence of wheat spindle streak mosaic virus among ten wheat cultivars and its effect on yield. Plant Dis. Rep. 58:522-525.