Use of Monoclonal Antibody to the Nonstructural Protein Encoded by the Small RNA of Tomato Spotted Wilt Tospovirus to Identify Viruliferous Thrips

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ABSTRACT

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Tomato spotted wilt tospovirus (TSWV) replicates in and is transmitted by the western flower thrips (*Frankliniella occidentalis*). Monoclonal antibodies (MAbs) were made to the nonstructural protein (NSs) encoded by the small RNA of TSWV. NSs is produced in thrips in which TSWV has replicated and potentially could transmit TSWV. MAbs were used in antigen coated plate enzyme-linked immunosorbent assay (ACP-ELISA) with the Zwitterionic detergent Empigen-BB (E-BB) at 0.1% (a.i.) in antibody dilution buffer to reduce nonspecific binding that results in high absorbance readings of control samples, which are commonly observed with insects in ACP-ELISA. With E-BB, a 10-fold difference in absorbance values was observed between adult thrips that fed on healthy plants and adult thrips that fed on virus-infected plants as larvae, compared to ACP-ELISA with Tween 20, in which there was only a threefold difference in absorbance values between the same samples of thrips. The utility of ACP-ELISA in identifying viruliferous thrips was compared with transmission of TSWV by thrips to *Petunia grandiflora*. The two assays were in agreement 92% of the time. The errors were divided: 6% occurred when ACP-ELISA detected thrips with NSs but the thrips were not identified as transmitters in the plant transmission assay, and 2% occurred when ACP-ELISA did not detect thrips that were positive in the plant transmission assay. These findings show that ACP-ELISA with E-BB is a conservative and useful tool for identifying viruliferous thrips and has potential for use in forecasting to manage TSWV epidemics.

Additional keyword: serology.

Tomato spotted wilt tospovirus (TSWV), the type species of the genus *Tospovirus* in the family Bunyaviridae, has a wide host range of at least 550 species of plants in over 75 families that include important and valuable crops (19). The virus is exclusively transmitted in a persistent manner by a number of thrips species, of which the western flower thrips (WFT), *Frankliniella occidentalis* Perg., is considered to be the most important (6). Adult thrips transmit TSWV only if the virus is acquired during the larval stage (8,14), with the majority of thrips becoming viruliferous (transmitters) in the second larval stage (19). Even though TSWV replicates in its thrips vector, transmission of TSWV by adults occurs intermittently for the duration of their lives (14,20).

Control of TSWV incidence by trying to control thrips with insecticides is ineffective because of the diverse host range of both virus and vector and the prolific reproduction of thrips (4). Yudin et al (21) indicated incidence of thrips was an important component of predicting TSWV incidence in lettuce, but without being able to identify the number of thrips in a population that are potential transmitters, thrips numbers were not reliable indicators of potential disease incidence. As a result, disease incidence early in the growing season had to be used as a predictor of final disease incidence. Determining the percentage of thrips in a population that are potential transmitters would allow vector populations to be used as a predictor of TSWV incidence in a preplanting forecasting system.

Transmission or serological assays have been used to identify viruliferous thrips from the field and greenhouse (1,4,5). Serological assays for detection of TSWV in thrips have used antibodies to TSWV structural proteins. These proteins are present in the digestive tract of any insect that has fed on a TSWV-infected plant (13). Many of these thrips will excrete the virus and never become transmitters; however, the thrips could be positive in a serological assay for detection of virus structural proteins. Including such thrips in estimates of thrips that are potential transmitters vastly overestimates the percentage of the vector population that could transmit TSWV. Assaying for viruliferous thrips by testing the ability of thrips to transmit TSWV to plants is useful but requires 2–3 days for symptoms to develop on plants after thrips have fed (1). This limits the utility of plant transmission assays for preplant forecasting.

Because TSWV replicates in those thrips that potentially may transmit TSWV, detection of a TSWV nonstructural protein (NS) could be used to differentiate thrips that have ingested virus but cannot transmit the virus from thrips in which TSWV has replicated and therefore could transmit the virus. Replication of TSWV in thrips results in readily detectable amounts of the NSs protein encoded by the small RNA, but the NSm protein encoded by the middle RNA is less frequently detected by immunoelectron microscopy (14; D. E. Ullman, *unpublished data*). In enzyme-linked immunosorbent assay (ELISA) with polyclonal antisera against NSs and the nucleocapsid (N) protein, Wijkamp et al (20) detected an increase of both proteins in thrips when the thrips acquired TSWV as larvae. However, ELISA absorbance values for an individual larvae using antiserum to NSs were very

low even after amplifying the initial enzyme-substrate reaction. In addition, the polyclonal antiserum produced had to be cross-adsorbed prior to use for detection of NSs. Only TSWV-viruliferous larvae, no adults, were tested in ELISA. These limitations suggest that ELISA with polyclonal antisera may not be suitable for differentiating thrips that are transmitters of TSWV from those that are nontransmitters. Nontransmitters would include thrips that have never fed on TSWV-infected tissue (virus-free) and thrips, such as adults, that may have fed on TSWV-infected plants but never become transmitters of the virus.

In this paper, we report the development and use of anti-NSs monoclonal antibodies (MAbs) for differentiating between thrips that are transmitters and nontransmitters. MAbs can be used in a standard ELISA format without additional amplification. In a preliminary study (D. E. Ullman and J. L. Sherwood, *unpublished data*), substantial nonspecific binding in antigen coated plate ELISA (ACP-ELISA) with virus-free thrips was observed. Wijkamp et al (20) cross-absorbed polyclonal antiserum produced to an expressed NSs protein with acetone-washed powder of healthy thrips to eliminate background in ELISA and Western blots. Zwitterionic detergents are known to reduce nonspecific binding in ELISA (2,15). Empigen-BB (E-BB), an alkyldimethylbetaine, restored antibody binding to meningococcal outer membrane proteins in Western blots when used with either transfer buffer (7) or in primary antibody solution (16). Allen et al (2) reported that the use of E-BB in the extraction buffer of parasitoid larval antigens from larvae of *Phyllonorycter blancardella* (F.) enhanced the reaction of antiserum to the parasitoid larvae and reduced nonspecific binding to the host larvae in double antibody sandwich ELISA (DAS-ELISA). In addition to the production of a MAb-based assay for detection of NSs, we investigated the use of E-BB in antibody dilution buffer to reduce nonspecific binding in ACP-ELISA so as to differentiate thrips that are potential transmitters and nontransmitters.

MATERIALS AND METHODS

Plant material and virus isolate. A TSWV isolate (formerly designated TSWV-L) collected from infected tomato on the Hawaiian island of Maui was used in all experiments. *Emilia sonchifolia* (L.) DC. ex Wight, *Datura stramonium* L., and *Petunia grandiflora* (L.) cv. Yellow Magic were grown from seed in greenhouses at the University of Hawaii-Manoa and used for maintenance of virus, transmission assays, and acquisition studies as described (13).

Production of anti-NSs MAb. A cloned cDNA to the NSs coding sequence was expressed in bacteria (14), and the protein was isolated from sodium dodecyl sulfate (SDS)-polyacrylamide gels in phosphate-buffered saline (PBS) (0.14 M NaCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.5 mM KCl, pH 7.5). The homogenized gel was centrifuged for 15 min at 10,000 *g*, and the NSs was recovered from the supernatant by a chloroform/methanol/water system (18). Fifty micrograms of NSs in PBS emulsified in Freund's complete adjuvant (Sigma Chemical Company, St. Louis) was used to immunize BALB/c mice. The NSs concentration was determined with Coomassie Plus Protein Assay Reagent (Pierce Chemical Co., Rockford, IL). Three subsequent immunizations were given at 10-day intervals using Freund's incomplete adjuvant (Sigma). After 20 days, a booster dose of 150 μg of NSs without adjuvant was injected. The spleen cells were fused with P3X63Ag8.653 myeloma cell line 48 h after the booster dose as previously reported (11). The screening and selection of cell lines was performed with NSs-coated magnetic beads (3). A clone designated 1C1A7 was the source of the MAb used in assay development. The cell line was grown in RPMI1640 (Mediatech, Inc., Herndon, VA) with 10% horse serum (HyClone Laboratories, Inc., Logan, UT). The supernatant was collected, and the antibody, an immunoglobulin (IgG2a), was precipitated from the culture supernatant with 50% ammonium sulphate and further purified using a protein-A column (Pierce). The antibody was stored in PBS with 0.02% sodium azide at 1 mg/ml after extensive dialysis against PBS.

Thrips acquisition of TSWV. The colonies of WFT, *F. occidentalis*, used in all experiments were maintained at the University of Hawaii-Manoa. Thrips were reared on green bean pods (*Phaseolus vulgaris* L.). To obtain viruliferous thrips, late first instar larvae were fed on TSWV-infected *E. sonchifolia* leaves for 48–72 h and then transferred to green bean pods to complete their development into adults in approximately 4–6 days. Virus-free adults were obtained by feeding the larvae on healthy *E. sonchifolia* leaves prior to transfer to green bean pods. Samples were obtained representing the following groups: 1) larvae fed on TSWV-infected *E. sonchifolia* as larvae and assayed as larvae directly following the 48–72 h of acquisition access feeding; 2) control larvae fed on virus-free *E. sonchifolia* and assayed as larvae at the same time as group 1; 3) larvae fed on TSWV-infected *E. sonchifolia* and assayed as adults; 4) larvae fed on virus-free *E. sonchifolia* and assayed as adults; and 5) adults fed on TSWV-infected *E. sonchifolia* for 48 h and assayed as adults. Thrips were placed in plastic vials containing PBS-PVP, pH 7.5 (2% polyvinylpyrrolidone), and express mailed to Oklahoma State University, Stillwater, for ACP-ELISA.

Effect of E-BB in antibody dilution buffer. The effect of E-BB (30% a.i.; Albright & Wilson Ltd., Whitehaven, England) and Tween 20 on reducing nonspecific binding in ACP-ELISA was compared by incorporating the detergents in the antibody dilution buffer. The antibody dilution buffer contained PBS, pH 7.5, with 0.1% bovine serum albumin (BSA) with either 0.05% Tween 20 (v/v) or 0.1% E-BB (a.i.). Although use of E-BB concentrations of 0.1–1.0% a.i. have been reported (2,7,16), 0.1% a.i. was used because of ease in handling the viscous material. For detection of NSs by ACP-ELISA in thrips, 20 thrips representing each of five groups as described above were homogenized in 1 ml of PBS-PVP, and wells of ELISA plates (Nunc MaxiSorp, PGC Scientific, Gaithersburg, MD) were coated with 50 μl each. Ten wells were used for each detergent treatment, and each treatment was replicated twice. Three independently collected thrips samples were tested. Control wells, used to zero the microplate reader, were coated with 1% BSA. After the samples were incubated overnight at 4 C, the plates were washed three times with PBS containing 0.05% Tween 20 (PBS-Tween), and blocked with 75 μl of 1% BSA in PBS for 2 h. Fifty microliters of anti-NSs MAb at 0.5 μg/ml in one of the two antibody dilution buffers was added to the wells, and the plates were incubated for 2 h at room temperature. After washing the wells with PBS-Tween four times, 50 μl of rabbit anti-mouse IgG-alkaline phosphatase (Sigma) at 0.2 μg/ml in antibody dilution buffer with the same detergent that was used to dilute the MAb was added to each well, and the plates were incubated for 2 h at room temperature. The wells were washed four times with PBS-Tween and 50 μl of substrate solution (1 mg/ml of *p*-nitrophenyl phosphate disodium in 1 M diethanolamine buffer that contained 0.5 mM $MgCl_2$ and 0.02% sodium azide) was added to each well. The absorbance was read at 405 nm with a V max computerized kinetic microplate reader (Molecular Devices, Palo Alto, CA). Absorbance values at 405 nm were taken at 0.5, 1, and 2 h after addition of substrate to determine the optimum time to read the plate. After finding the optimum time of incubation at room temperature after addition of substrate, the effect of overnight incubation at 4 C after the addition of substrate on absorbance values obtained was examined.

Thrips transmission of TSWV. Adult thrips that fed on TSWV-infected *E. sonchifolia* as larvae were tested for their ability to transmit TSWV as adults, using a modification of the plant transmission assay developed by Wijkamp and Peters (19). A leaf disk (1 cm diameter) of *P. grandiflora* cv. Yellow Magic (Harris Seeds, Rochester, NY) freshly cut with a cork borer was placed in a 1.5-ml centrifuge tube along with a piece of Whatman #2 filter paper (Clifton, NJ) previously dried over desiccant. Thrips were starved for 1–2 h and then individually fed on separate leaf disks for 24 h. Thrips were then removed and individually stored in the buffer described above for ACP-ELISA, and the leaf disk was placed on water for the development of localized lesions. Symptoms on leaf disks were visually scored at 24-h intervals

for 72 h. Virus-free thrips were included as controls. The experiment was replicated four times with either 25, 50, 90, or 100 adult thrips.

ACP-ELISA of thrips. The ACP-ELISA was used to detect NSs in individual larvae and adult thrips. An individual thrips was ground in 50 μl of PBS-PVP buffer in a porcelain mortar with a disposable glass tube, and the sample was placed in a well of an ELISA plate. Negative controls of virus-free thrips also were run. The plates were incubated at 4 C overnight, and the procedure was continued as described above. The antibody dilution buffer with E-BB at 0.1% (a.i.) was used. When results of the plant transmission assay and ACP-ELISA for the same thrips were compared, a positive/negative threshold of absorbance at 405 nm of 0.100 was used. This threshold was based on results obtained from the experiments with groups of thrips and absorbance values obtained with known virus-free thrips assayed as controls. For the experiments using 25, 50, 90, or 100 thrips, controls of 5, 9, 7, or 24 thrips, respectively, were used. The average absorbance at 405 nm plus four standard deviations ranged from 0.043 to 0.091, so 0.100 was used across all experiments. A two by two *G* test for independence (9) was used to test whether results for identifying viruliferous thrips by plant transmission assay and ACP-ELISA were independent.

RESULTS

Effect of E-BB in antibody dilution buffer. The use of E-BB reduced the nonspecific binding to thrips homogenates tested in ACP-ELISA (Table 1). With E-BB, a 10-fold difference in absorbance values was observed between adult thrips fed on healthy plants and adult thrips fed on virus-infected plants as larvae. With Tween 20, there was only a threefold difference in absorbance values between adult thrips fed on healthy plants and adult thrips fed on virus-infected plants as larvae. The difference was 20- and fivefold with E-BB and Tween 20, respectively, with larvae fed on healthy plants and larvae assayed after feeding on TSWV-infected plants for 48–72 h. Absorbance values in ACP-ELISA for adult thrips fed on TSWV-infected plants as adults was about twice the value of thrips fed on healthy plants when E-BB was used; small amounts of NSs ingested during feeding were detected. The corresponding values with Tween 20 showed no apparent difference in the absorbance values. Because E-BB effectively reduced the nonspecific binding to thrips homogenates tested in ACP-ELISA compared to Tween 20, E-BB was used in the remaining assays.

Effect of substrate incubation time on absorbance values. Reading absorbance values 1 h after the addition of substrate permitted differentiation between virus-free thrips and thrips fed on virus-infected plants as either adults or larvae (Table 2). After 1 h, there was a disproportionate increase in color development in samples of thrips that would not be transmitters compared to samples of larvae or adults that would be transmitters. When after the addition of substrate plates were incubated at 4 C overnight compared to 1 h at room temperature, groups of thrips that could be transmitters versus nontransmitters could be readily differentiated (Table 3). As in the other assays, the difference was more apparent when E-BB was used than when Tween 20 was used.

Sensitivity of ACP-ELISA for detection of NSs. The ACP-ELISA was conducted on gel-isolated NSs, obtained to use as an immunogen, to determine a limit on the amount of NSs that could be detected by ACP-ELISA. Two wells were each coated with one of a series of twofold dilutions of NSs ranging in concentrations from 1,000 to 0.122 ng/ml. One hour after the addition of substrate the plate was read. Average absorbance readings at 405 nm for NSs at 0.122, 0.244, 0.488, and 0.976 ng/ml were 0.030, 0.126, 0.226, and 0.436, respectively, compared to the BSA control used to zero the plate. Absorbance readings at 405 nm for increasing NSs concentrations continued to approximately double as the NSs concentration increased to 7.8 ng/ml. At 7.8 ng/ml, the absorbance reading at 405 nm after 1 h was 2.151. Between 7.8 and 250 ng/ml, the absorbance reading at 405 nm increased about 10% as the NSs concentration was doubled. The absorbance readings for NSs concentrations between 250 and 1,000 ng/ml was 3.715 ± 0.198. Thus, the lower limit of detection of NSs was about 0.244 ng/ml. The absorbance values obtained in ACP-ELISA over a range of concentrations was typical of standard curves obtained in ELISA.

Comparison of ACP-ELISA and transmission assay for detection of viruliferous thrips. Based on the results of the four replica-

TABLE 1. Comparison of the effect of Empigen-BB and Tween 20 in the antibody dilution buffer on detection of tomato spotted wilt tospovirus (TSWV) nonstructural protein (NSs) in thrips by antigen coated plate enzyme-linked immunosorbent assay

	Absorbance (405 nm)[b]					
	Empigen-BB			Tween 20		
Sample[a]	1	2	3	1	2	3
Adults fed on TSWV-infected plants as adults	0.135 ± 0.009	0.180 ± 0.016	0.150 ± 0.010	0.380 ± 0.067	0.450 ± 0.082	0.400 ± 0.073
Adults fed on TSWV-infected plants as larvae	0.920 ± 0.032	0.980 ± 0.051	1.050 ± 0.071	1.200 ± 0.142	1.450 ± 0.126	1.270 ± 0.136
Adults fed on healthy plants	0.095 ± 0.007	0.090 ± 0.012	0.080 ± 0.008	0.495 ± 0.116	0.460 ± 0.091	0.495 ± 0.102
Larvae fed on TSWV-infected plants	1.275 ± 0.041	1.350 ± 0.098	1.875 ± 0.128	1.870 ± 0.195	1.830 ± 0.189	2.235 ± 0.272
Larvae fed on healthy plants	0.075 ± 0.004	0.065 ± 0.008	0.045 ± 0.008	0.400 ± 0.087	0.430 ± 0.076	0.375 ± 0.049

[a]Each sample of 20 thrips was homogenized in 1 ml of phosphate-buffered saline with 2% polyvinylpyrrolidone (pH 7.5), and 20 wells were coated with 50 μl each.
[b]Mean absorbance values ± standard deviation for 20 wells, taken after 1 h.

TABLE 2. Effect of Empigen-BB and Tween 20 in the antibody dilution buffer on detection of tomato spotted wilt tospovirus (TSWV) nonstructural protein (NSs) in thrips by antigen coated plate enzyme-linked immunosorbent assay at different times after addition of substrate

	Absorbance (405 nm) at different times (h)[b]					
	Empigen-BB			Tween 20		
Sample[a]	0.5	1	2	0.5	1	2
Adults fed on TSWV-infected plants as adults	0.115 ± 0.012	0.180 ± 0.016	0.250 ± 0.017	0.275 ± 0.079	0.450 ± 0.082	0.625 ± 0.086
Adults fed on TSWV-infected plants as larvae	0.450 ± 0.048	0.980 ± 0.051	1.150 ± 0.054	0.850 ± 0.118	1.450 ± 0.126	2.150 ± 0.127
Adults fed on healthy plants	0.055 ± 0.014	0.090 ± 0.012	0.185 ± 0.018	0.280 ± 0.088	0.460 ± 0.091	0.830 ± 0.089
Larvae fed on TSWV-infected plants	0.950 ± 0.087	1.350 ± 0.098	1.650 ± 0.096	0.905 ± 0.196	1.830 ±0.189	2.650 ± 0.198
Larvae fed on healthy plants	0.040 ± 0.007	0.065 ± 0.008	0.185 ± 0.013	0.240 ± 0.042	0.430 ± 0.076	0.975 ± 0.079

[a]Twenty thrips were homogenized in 1 ml of phosphate-buffered saline with 2% polyvinylpyrrolidone (pH 7.5), and 20 wells were coated with 50 μl each.
[b]Mean absorbance values ± standard deviation for 20 wells.

TABLE 3. Effect of incubation in enzyme substrate at 4 C overnight (ON-4C) versus 1 h at room temperature (1h-RT) on antigen coated plate enzyme-linked immunosorbent assay for detection of tomato spotted wilt tospovirus (TSWV) nonstructural protein (NSs) in thrips

	Absorbance (405 nm)[b]			
	Empigen-BB		Tween 20	
Sample[a]	1h-RT	ON-4C	1h-RT	ON-4C
Adults fed on TSWV-infected plants as adults	0.180 ± 0.016	0.205 ± 0.014	0.450 ± 0.082	0.395 ± 0.078
Adults fed on TSWV-infected plants as larvae	0.980 ± 0.051	0.985 ± 0.042	1.450 ± 0.126	1.580 ± 0.112
Adults fed on healthy plants	0.090 ± 0.012	0.087 ± 0.011	0.460 ± 0.091	0.380 ± 0.084
Larvae fed on TSWV-infected plants	1.350 ± 0.098	1.470 ± 0.078	1.830 ± 0.189	1.740 ± 0.175
Larvae fed on healthy plants	0.065 ± 0.008	0.075 ± 0.003	0.430 ± 0.076	0.325 ± 0.072

[a]Twenty thrips were homogenized in 1 ml of phosphate-buffered saline with 2% polyvinylpyrrolidone (pH 7.5), and 20 wells were coated with 50 μl each.
[b]Mean absorbance values ± standard deviation for 20 wells.

TABLE 4. Identification of tomato spotted wilt tospovirus (TSWV) viruliferous thrips by antigen coated plate enzyme-linked immunosorbent assay (ACP-ELISA) (absorbance at 405 nm) using monoclonal antibody to TSWV nonstructural protein (NSs) and by thrips transmission to *Petunia grandiflora*

	Experimental group of thrips[a,b]			
	1	2	3	4
Absorbance values for thrips positive in ACP-ELISA	0.372 +[c] 0.168 +	0.713 + 0.414 + 0.214	1.288 + 1.073 + 1.016 1.010 + 0.859 + 0.808 + 0.689 + 0.674 + 0.629 + 0.411 0.364 + 0.295 0.294 0.270 + 0.263 + 0.216 + 0.134 + 0.131 0.130	3.000 + 2.077 + 2.064 1.728 1.610 + 1.425 + 1.372 + 0.765 + 0.724 + 0.712 0.612 0.521 + 0.421 + 0.421 0.412 0.402 + 0.318 0.311 0.299 + 0.299 + 0.289 0.278 0.218 +
No. of thrips negative in ACP-ELISA ($A_{405nm} < 0.100$)	23	47	71	77
No. of thrips positive in plant transmission assay, but negative in ACP-ELISA	1 (0.006)[d]	1 (0.059)	2 (0.085, 0.000)	1 (0.000)
Total number of thrips assayed	25	50	90	100

[a]Thrips were fed on TSWV-infected *Emilia sonchifolia* plants as larvae, fed on healthy *E. sonchifolia* until adults, and then assayed on leaf disks of *P. grandiflora*.
[b]Individual thrips were homogenized in 50 μl of phosphate-buffered saline (PBS) with 2% polyvinylpyrrolidone and coated to the plate. Empigen-BB was used at 0.1% in antibody dilution buffer (PBS with 0.1% bovine serum albumin, pH 7.5). Absorbance was measured at 405 nm after 1 h. Positive/negative absorbance threshold in ACP-ELISA at 405 nm was 0.100.
[c]+, thrips was positive in transmission assay to *P. grandiflora*.
[d]Absorbance value in ACP-ELISA.

tions using 25, 50, 90, or 100 thrips, the ACP-ELISA and the plant transmission assay were similar in identifying viruliferous thrips (Table 4). In a G test for independence, the two assays showed close agreement. The G test indicated that the results of the two tests were not independent ($G = 97.72$; 1 df; $P < 0.0001$). The two assays were in agreement 92% of the time. The errors were divided: 6% occurred when ACP-ELISA identified thrips as potential transmitters that were not identified as transmitters in the plant transmission assay, and 2% occurred when ACP-ELISA did not detect individuals that transmitted TSWV in the plant transmission assay.

DISCUSSION

ACP-ELISA with MAbs to the NSs protein of TSWV and the Zwitterionic detergent E-BB is an efficient and economical method to screen a large number of thrips for their potential to transmit TSWV. The inclusion of detergents in serological reagents to reduce nonspecific binding is common. The development of the ACP-ELISA described here was initiated with four MAbs produced against NSs with no apparent cross-reactivity to healthy plant tissue in ACP-ELISA (3). Because of the nonspecific binding of antibody to thrips homogenate in ACP-ELISA when Tween 20 was used, only one of the four MAbs, MAb 1C1A7 used in this study, made possible differentiation between samples of nonviruliferous or viruliferous thrips (data not shown). The polyoxyethylene detergent Tween 20 commonly used in ELISA and Western blot assay did not minimize the nonspecific binding, which was needed if individual viruliferous and nonviruliferous thrips were to be reliably differentiated.

The effect or function of detergents used in serological assays is not predictable or well understood (10,12,16,17). Zwitterionic detergents improved results obtained in Western blots and ELISA (2,7,16). Wedge et al (16) concluded that Zwitterionic detergents, like CHAPS and E-BB, enhanced the binding of antibody to antigen and suggested that these detergents may be employed when antibodies with low or medium titers are used. Vogt et al (15) and Allen et al (2) observed that Zwitterionic detergents prevented the nonspecific binding of biotinylated antibody when used in double antibody sandwich ELISA with enzyme amplification. We used E-BB in this study because the detergent is economical compared to other Zwitterionic detergents and reduced the nonspecific binding to thrips homogenates in ACP-ELISA. However, because final ACP-ELISA values were not appreciably higher when E-BB instead of Tween 20 was used, we cannot conclude, as Wedge et al (16) suggested, that E-BB enhances the reaction between antigen and antibody. Whether E-BB may renature the antigens that are denatured by SDS (16) or reduce the nonspecific binding of primary and secondary antibodies (2,15) is unclear. However, use of E-BB clearly reduced the nonspecific signal of MAbs made to NSs to nonviruliferous thrips compared to Tween 20.

ACP-ELISA with E-BB in the antibody dilution buffer is a rapid and simple assay that differentiates thrips that are nontransmitters from transmitters. This is supported by the close agreement found between the results of ACP-ELISA and plant transmission assay. The greatest amount of error (6%) was in ACP-ELISA identifying thrips as potential transmitters that were not detected in the plant transmission assay. This result is to be expected because plant inoculation by thrips is subject to many biological factors (i.e., whether the thrips feed appropriately on the leaf disk). In contrast, ACP-ELISA was free of this type

of variable, and NSs was readily detected, thus identifying thrips that could transmit TSWV to *P. grandiflora*.

Error in identifying potential transmitters that did not transmit in the plant transmission assay may be beneficial because most of the potential transmitters in the population would be detected. Thus, ACP-ELISA estimates of viruliferous thrips are very conservative and valuable for prediction purposes. A 2% error occurred when ACP-ELISA did not detect individuals that transmitted TSWV in the plant transmission assay. This type of error is more serious, because the ACP-ELISA missed individuals that were viruliferous. This may have occurred for several reasons. Titers of NSs do fluctuate in the thrips and may have dropped prior to ACP-ELISA in the few individuals where this error occurred, NSs antigens may have degraded in the thrips during shipment so the protein was not detected by ACP-ELISA, or thrips feeding damage on leaf disks may have been mistakenly scored as positive for TSWV transmission. This type of error involved only a very small percentage of the total number of thrips assayed and should not limit the utility of ACP-ELISA for screening thrips populations for potential transmitters. ACP-ELISA certainly permits screening of thrips in less time with less labor than plant transmission assays. Furthermore, ACP-ELISA is economical and less complicated than ELISA that used enzyme amplification systems previously reported to differentiate thrips that are potential transmitters from nontransmitters (20).

The utility of pooling thrips or applying this system to field captured insects has not been examined. Appropriate field sampling methods and refinements for testing very large numbers of thrips should be feasible. Yudin et al (21) found that disease incidence in lettuce at harvest was significantly associated with early disease incidence and early thrips abundance although thrips numbers could not be used as a reliable predictor of TSWV epidemics. The ability to readily identify viruliferous thrips will allow development of effective models to predict disease prior to crop planting and permit growers to avoid epidemics and concomitant economic losses. The utility of ACP-ELISA for NSs in developing a forecasting system for TSWV epidemics will be evaluated in the future.

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