Comparative Biological and Serological Properties of Four Strains of Zucchini Yellow Mosaic Virus

H. L. WANG, Former Graduate Research Assistant, Department of Plant Pathology, Cornell University New York State Agricultural Experiment Station; D. GONSALVES and R. PROVVIDENTI, Professors, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456; and T. A. ZITTER, Associate Professor, Department of Plant Pathology, Cornell University, Ithaca, NY 14850

ABSTRACT

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Four strains of zucchini yellow mosaic virus (ZYMV) from Connecticut (CT), Florida (FL), France (WK), and Taiwan (TW) were partially characterized and compared. Host ranges of the four strains were similar but they differed in the ability to infect specific hosts. All ZYMV strains were indistinguishable in SDS-immunodiffusion tests. After cross-absorption with antigens of ZYMV-CT and -FL, antiserum to ZYMV-TW reacted with ZYMV-TW but not with ZYMV-CT, -FL, or -WK in enzyme-linked immunosorbent assays. Serological comparisons revealed that ZYMV was related to watermelon mosaic 2 (WMV-2), pea mosaic (PMV), bean common mosaic (BCMV), clover yellow vein (CYVV), and bean yellow mosaic (BYMV) viruses. The relatedness of ZYMV to these viruses was shown only with antisera produced to the ZYMV strains and varied depending on the length of time after immunization that antisera was collected. Purified virus yields of the four strains of ZYMV ranged from 6 to 19 mg of virus per 100 g of infected tissue with a 260/280 nm absorbance ratio range of 1.22 to 1.27. Two aphid species, *Myzus persicae* and *Aphis gossypii*, transmitted ZYMV-CT, ZYMV-FL, and ZYMV-TW at different efficiencies but failed to transmit ZYMV-WK.

Ten years after its characterization in Italy and France (13), zucchini yellow mosaic virus (ZYMV) remains one of the most widespread and destructive viral agents affecting cucurbits. The apparently rapid spread in the 1980s of this virus in many countries suggested a long distance distribution via infected seeds. However, studies involving a large number of summer squash (*Cucurbita pepo* L.) and melon (*Cucumis melo* L.) plants from seeds of severely infected plants have failed to conclusively prove this avenue of dissemination (3,20). In the same period, relatively few studies have been conducted to identify overwintering or oversummering noncucurbitaceous hosts of this virus. In one of these studies, ZYMV was detected in a few naturally infected weeds and demonstrated that it can be seedborne in *Ranunculus sardous* Crantz at a very low rate (1). The rapid detection of ZYMV in several regions of the world can be attributed mainly to an effective international cooperation among researchers and to the antisera provided by V. Lisa and H. Lecoq (13).

Present address of first author: Fengshan Tropical Horticultural Experiment Station, TARI, Kaohsiung 830, Taiwan, R.O.C.

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For a newly characterized virus, ZYMV has already displayed a remarkable variability (13), suggesting that it has existed for a long time. A number of isolates have been characterized that are able to incite a variety of symptoms, to infect specific hosts (including resistant genotypes), or are not transmissible by aphids (9,13). However, most of these isolates were proven to be closely related serologically. This difference among isolates of ZYMV is typified by the two major strains identified in the United States since 1982 (19). The Connecticut strain (ZYMV-CT) and the Florida strain (ZYMV-FL) are easily recognized by the differences in incubation periods, symptoms caused in summer squash and cucumber (*C. sativus* L.), and the differential reaction of certain watermelons (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) from Zimbabwe (18). Conversely, serologically they appear to be identical (19), and genes for resistance in cucumber, squash, melon, and a wild watermelon (*C. colocythis* (L.) Schrader) appear to function equally well for both strains (15).

The objectives of this research were to compare the biological and serological properties of these American strains with two diverse strains from France and Taiwan and to determine serological relationships of ZYMV strains with other well-known potyviruses, such as bean common mosaic (BCMV), clover yellow vein (CYVV), pea mosaic (PMV), and watermelon mosaic 2 (WMV-2). Our results show that host ranges of ZYMV strains were similar, but these strains incited some different reactions on indicator plants and the mild strain of ZYMV was not transmitted by aphids. Additionally, ZYMV-TW antiserum specifically reacted to only ZYMV-TW in enzyme-linked immunosorbent assay (ELISA) after absorption of antisera with ZYMV-FL or -CT. This latter property proved valuable in evaluating cross-protection experiments in Taiwan (25).

MATERIALS AND METHODS

Viruses. The Florida and Connecticut strains of ZYMV, PMV, BCMV, bean yellow mosaic virus (BYMV), a watermelon strain of papaya ringspot virus (PRSV-W), and CYVV were from our laboratory. A Florida strain of WMV-2 (FC-1656) and a mild strain of ZYMV (ZYMV-WK) were supplied by D. Purcifull (University of Florida) and H. Lecoq (INRA, France), respectively. The Taiwan strain of ZYMV (ZYMV-TW) was from C. H. Huang (Taiwan Agriculture Research Institute, Taiwan). ZYMV, PRSV-W, and WMV-2 were maintained in squash cv. Zucchini Elite, and BCMV, CYVV, BYMV, and PMV in pea, *Pisum sativum* L. 'Ranger'.

Host range. At least 10 test plants of each species or cultivar belonging to the families Amaranthaceae, Caricaceae, Leguminosae, and Solanaceae were inoculated by rubbing inocula on leaves previously dusted with 600-mesh Carborundum. Inocula were derived from freshly harvested leaves of greenhouse-grown infected zucchini plants ground in 0.01 M potassium phosphate buffer, pH 7.0. The plants were maintained in a greenhouse at 25–30 C. Virus symptoms on plants were recorded 2 wk after inoculation and then at regular intervals during the next 4 wk. All plants not showing symptoms were assayed for virus infection by inoculation of indicator hosts or by ELISA (2).

Aphid transmission. Green peach aphids (*Myzus persicae* (Sulzer)) and cotton aphids (*Aphis gossypii* Glover) reared on cucumber were fasted for a 2-hr preacquisition period and then transferred to symptomatic zucchini leaves infected with one of the four ZYMV strains (6–10 days after inoculation). After a 5-min access feeding period, five aphids were placed on each of 10 healthy caged Zucchini Elite plants,

kept overnight, then killed by insecticide (DDVP). Test plants were maintained in a greenhouse, observed for symptom development over a 3-wk period, and then assayed by ELISA for the presence of ZYMV. Healthy zucchini leaves were used as controls. This test was repeated twice.

Measurement of relative virus titers in squash. Relative virus titers among ZYMV strains in infected zucchini plants were determined by counting the number of local lesions incited on inoculated *Chenopodium quinoa* Willd. Thirty zucchini plants inoculated with ZYMV were grown at 25 C in a growth chamber, and young leaves from each of five inoculated plants were harvested at 4, 6, 8, 10, 12, and 14 days, respectively, after inoculation. Leaves harvested from systemically infected plants were cut into small pieces with a razor blade, mixed thoroughly, and ground in 0.01 M potassium phosphate buffer (1 g/50 ml), pH 7.0. The crude sap was passed through two layers of cheesecloth and rubbed onto five leaves of each of five *C. quinoa* plants. Local lesions were counted about 2 wk after inoculation.

Purification of ZYMV strains. Leaf tissue of ZYMV-infected Zucchini Elite was harvested at the optimal period after inoculation according to the results of the virus titer experiments. Each virus strain was purified according to the method of Gonsalves and Ishii (4) as modified by Huang et al (5). Briefly, the procedure involved grinding tissue in high salt buffer, precipitating the virus with polyethylene glycol, and isolating viral zones after cesium sulfate density gradient centrifugation. Final virus preparations were resuspended in 0.05 M potassium phosphate, pH 7.5. Purities and yields of the virus preparations were determined by spectrophotometry without correction for light scattering.

Antisera production. Antisera to ZYMV strains were produced in white New Zealand rabbits. Each rabbit was injected subcutaneously with a purified virus preparation emulsified (1:1) with Freund's complete adjuvant (first injection) and with Freund's incomplete adjuvant (second and third injections). Injections at weekly intervals consisted of 1 mg of virus in 1 ml of 0.05 M potassium phosphate buffer, pH 7.5. Normal sera were obtained from each rabbit before immunization. The rabbits were bled weekly for 12–14 wk, starting 2 wk after the final injection. Antisera titers were determined by SDS-immunodiffusion tests in media containing 0.8% Noble agar, 0.5% SDS, and 1.0% sodium azide (21). Crude leaf extracts in 1% SDS were used as antigen sources. An antiserum to a Florida isolate of WMV-2 was obtained from D. E. Purcifull. Antisera to coat protein and 54-K nuclear inclusion of a PV-2 isolate of BYMV (BYMV-PV-2, now considered a distinct entity from BYMV as pea mosaic virus [17]), BCMV, and CYVV were provided by C. A. Chang (Taiwan Agriculture Research Institute, Taiwan). An antiserum to ZYMV-TW, obtained 6 wk after final immunization, was supplied by C. H. Huang (Taiwan Agriculture Research Institute, Taiwan), and an antiserum to cytoplasmic inclusion of ZYMV-CT was

Table 1. Host reactions of four strains[a] of zucchini yellow mosaic virus (ZYMV)

	Symptoms[b] of ZYMV strains							
	Inoculated leaves				Systemic leaves			
Test plant	CT	FL	WK	TW	CT	FL	WK	TW
Amaranthaceae								
Gomphrena globosa	cll	cll	cll	cll	sm,y	sm,hd	mm	sm,sd
Caricaceae								
Carica papaya	–[c]	–	–	–	–	–	–	–
Chenopodiaceae								
Chenopodium amaranticolor	nll	cll	cll	cll	–	–	–	–
C. quinoa	nll	cll	cll	cll	–	–	–	–
Cucurbitaceae								
Citrullus lanatus	sl	sl	sl	sl	m	m	sl	m
Cucumis melo								
Rocky Ford	nll	sl	sl	nll	sm,w	sm	sl	sm
Saticoty	nll	sl	sl	nll	sm,w	sm	sl	sm
PD 23	nll	sl	sl	nll	sm,w	sm	sl	sm
C. sativus								
Marketer	sl	sl	sl	sl	sm	sm	sl	sm
Lemon	sl	sl	sl	sl	sm	sm	sl	sm
Chain #3	nll	nll	sl	nll	nll	nll	sl	nll
Taichung Mou Gua	sl	sl	sl	sl	–	–	–	–
Cucurbita pepo								
Zucchini Elite	nll	sl	sl	cll	sm,y	sm,hd	mm	sm,sd
Zucchini President	nll	sl	sl	cll	sm,y	sm,hd	mm	sm,sd
Luffa acutangula	sl	sl	sl	ics	mm	mm	sl	ics
Leguminosae								
Glycine max	–	–	–	–	–	–	–	–
Phaseolus vulgaris								
Black Turtle #1	–	–	–	–	–	–	–	–
Black Turtle #2	nll	–	nll	nll	–	–	–	–
Red kidney	cll	–	cll	cll	–	–	–	–
Pisum sativum								
Ranger	sl	sl	sl	sl	–	–	–	–
Vigna angularis	–	–	–	–	–	–	–	–
V. unguiculata	–	–	–	–	–	–	–	–
Solanaceae								
Datura stramonium	–	–	–	–	–	–	–	–
Lycopersicon esculentum	–	–	–	–	–	–	–	–
Nicotiana benthamiana	sl	–	–	sl	–	–	–	–
N. tabacum	–	–	–	–	–	–	–	–

[a] Strains are designated as CT (Connecticut), FL (Florida), WK (French mild), and TW (Taiwan).
[b] cll = Chlorotic local lesion; hd = hour-glass distortion; ics = irregular chlorotic spot; m = mild mosaic; nll = local lesion; sd = shoestring distortion; sl = symptomless infection; sm = severe mosaic; w = wilting; and y = yellowing. The reactions were confirmed by ELISA tests.
[c] – = No symptom and negative reaction in ELISA test.

prepared in our laboratory (19).

Serology. Serological comparisons were done in SDS-immunodiffusion tests with the four strains of ZYMV and PRSV-W, WMV-2, PMV, BYMV, BCMV, CYVV, and their respective antisera. The ZYMV-TW antiserum was cross-absorbed with crude antigens from ZYMV-CT and ZYMV-FL according to the method of Huang et al (5). One milliliter of antiserum was mixed with 20 ml of a crude extract prepared from 10 g of fresh infected leaf tissue ground in 0.1 M potassium phosphate buffer, pH 7.0, and brought up to a final volume of 20 ml. The preparation was incubated at 37 C for 1 hr, then cooled in ice for an additional hour. Precipitates were discarded after a low-speed centrifugation at 3,000 *g* for 10 min. Immunoglobulins were purified using a DEAE sephacel column (Pharmacia Co., Piscataway, NJ) from noncross-absorbed and cross-absorbed ZYMV-TW antisera, and the aliquots were conjugated to alkaline phosphatase (2). Direct double-antibody sandwich (DAS) ELISA (2) and indirect ELISA (8,14) tests were conducted.

For direct DAS-ELISA, microtiter plates were coated with 1.25 μg/ml of immunoglobulin for 2 hr at 37 C. Antigen sources were ground (1 g/20 ml) in 0.01 M potassium phosphate buffer, pH 7.5, and incubated overnight at 4 C, and the conjugated immunoglobulins were added at various dilutions (1/500, 1/1,000, 1/1,500, 1/2,000, 1/2,500, and 1/3,000) and incubated for 3 hr at 37 C. For indirect ELISA, leaf tissue was triturated in coating buffer (2) at a 1:20 dilution (w/v) and incubated overnight at 4 C, and virus-specific immunoglobulins were used at decreasing dilutions starting from 1 μg/ml. Goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma Co., St. Louis, MO) was used at a 1:1,000 dilution and incubated for 1.5 hr at 37 C. Reactions were measured at A_{405nm} absorbance on a MicroElisa Reader (MR 580, Dynatech Instruments, Inc., Torrance, CA) 30 min after addition of substrate. An ELISA reaction was considered positive if it exceeded the mean plus four standard deviations of the healthy tissue controls and had a minimum OD_{405nm} reading of 0.1.

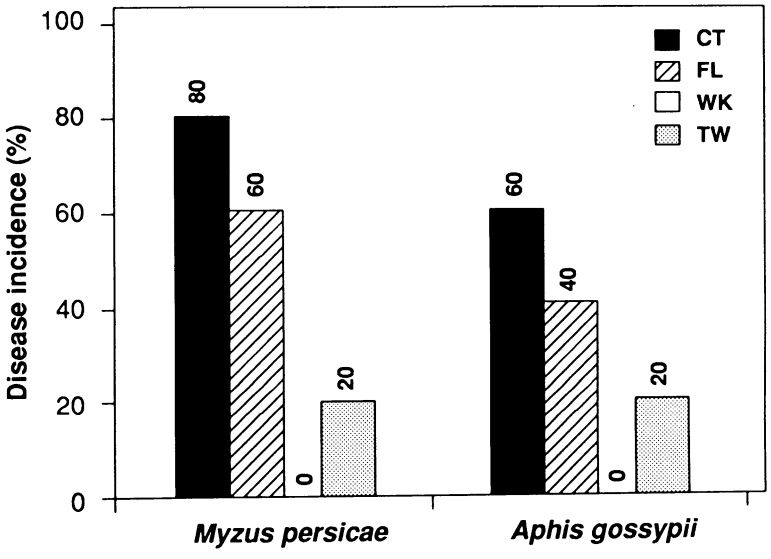

Fig. 1. The transmissibility of four strains of zucchini yellow mosaic virus (ZYMV) by *Myzus persicae* and *Aphis gossypii*. CT, FL, WK, and TW refer to ZYMV Connecticut, Florida, French mild, and Taiwan strains, respectively. Ten plants were used in each treatment, and disease incidence was the average of the two experiments.

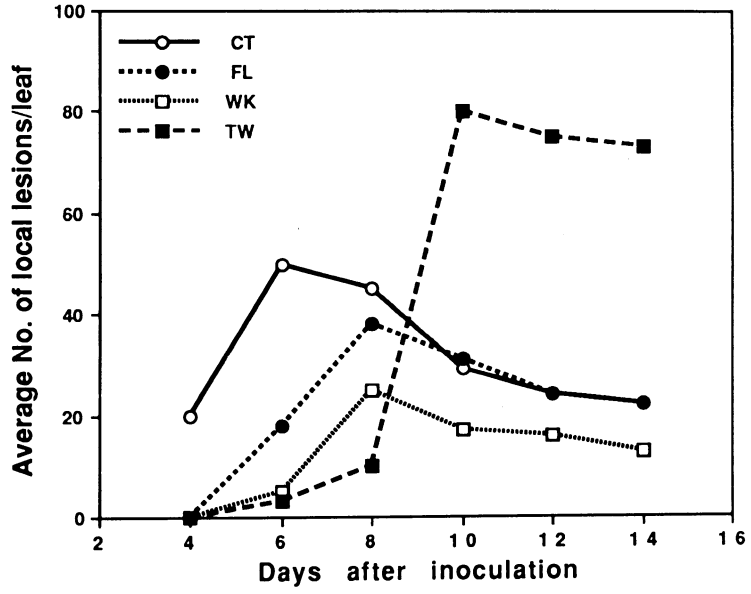

Fig. 2. Comparison of relative local lesion production on *Chenopodium quinoa* after inoculation with four strains of zucchini yellow mosaic virus (ZYMV). CT, FL, WK, and TW were ZYMV Connecticut, Florida, French mild, and Taiwan strains, respectively.

RESULTS

Host range. Eighteen plant species belonging to six different families were tested. From the data shown in Table 1, it is evident that the four strains varied in host reactions. ZYMV-WK differed from the other strains by inducing a mild mosaic to symptomless systemic infection in cucurbits. Conversely, ZYMV-CT, ZYMV-FL, and ZYMV-TW incited more severe symptoms in the same hosts. ZYMV-CT induced local necrotic lesions on *C. amaranticolor* Coste & Reyn. and *C. quinoa*, whereas ZYMV-FL, -WK, and -TW induced local chlorotic lesions. ZYMV-CT, -WK, and -TW incited local necrotic lesions on Black Turtle #2 bean and chlorotic lesions on red kidney bean, but ZYMV-FL did not infect these cultivars. *Nicotiana benthamiana* Domin. responded to ZYMV-CT and ZYMV-TW with only symptomless local infection.

Aphid transmission. Using two aphid species, transmission rates of ZYMV-CT and -FL were at least twice that of ZYMV-TW. ZYMV-WK was not transmitted by either aphid species (Fig. 1).

Relative virus titers of ZYMV-CT, -FL, -TW, and -WK in zucchini. Virus titers differed among the four strains in zucchini plants at various intervals after inoculation (Fig. 2). ZYMV-CT caused the highest number of local lesions 5–7 days after inoculation, after which the titer leveled off. However, ZYMV-TW did not reach maximal titer until 10 days after inoculation, but the titer was much higher than that of the other strains. ZYMV-FL and -WK incited the highest number of local lesions 7–9 days after inoculation. Maximal titer of ZYMV-WK was lowest of all strains.

Purification of virus isolates. The yields of purified virus from infected zucchini plants varied among the strains

Table 2. Antisera titers[a] to four strains of zucchini yellow mosaic virus (ZYMV)[b] and their serological relationships with other potyviruses as determined in SDS-immunodiffusion tests

No. of bleed	ZYMV-CT						ZYMV-FL						ZYMV-WK						ZYMV-TW					
	Titer	W-2	PM	BYM	CYV	BCM	Titer	W-2	PM	BYM	CYV	BCM	Titer	W-2	PM	BYM	CYV	BCM	Titer	W-2	PM	BYM	CYV	BCM
1	0	−[c]	−	−	−	−	16	+	−	−	−	−	2	−	−	−	−	−	0	−	−	−	−	−
2	0	−	−	−	−	−	16	+	−	−	−	−	2	−	−	−	−	−	2	−	−	−	−	−
3	4	+	−	−	−	−	32	+	−	−	−	−	4	−	−	−	−	−	8	−	−	−	−	−
4	8	+	−	−	−	+	32	+	−	−	+	−	8	−	−	−	−	−	8	−	−	−	−	−
5	16	+	+	−	−	+	32	+	−	−	+	−	8	−	−	−	−	−	8	−	−	−	−	−
6	16	+	+	−	−	+	32	+	−	−	+	−	8	−	−	−	−	−	16	−	−	−	−	−
7	32	+	+	−	−	+	32	+	+	−	+	−	8	−	−	−	−	−	16	−	−	−	−	−
8	8	+	+	−	−	+	32	+	+	−	+	−	8	−	−	−	−	−	8	+	+	−	−	+
9	16	+	+	−	+	+	32	+	+	−	+	−	8	−	+	−	−	−	8	+	+	−	−	+
10	16	+	+	+	+	+	16	+	+	−	+	+	4	−	+	−	−	−	8	+	+	−	−	+
11	16	+	+	+	+	+	32	+	+	−	+	+	8	−	+	−	−	−	16	+	+	−	−	+
12	32	+	+	+	+	+	16	+	+	−	+	+	8	−	+	−	−	−	16	+	+	−	−	+
13	32	+	+	+	+	+	16	+	+	−	+	+							16	+	+	−	−	+
14	32	+	+	+	+	+													16	+	+	−	−	+

[a] Antisera titers were $1/x$; W-2 = watermelon mosaic virus; PM = pea mosaic virus; CYV = clover yellow vein virus; and BCM = bean common mosic virus.
[b] Antisera to strains Connecticut (CT), Florida (FL), French mild (wk), and Taiwan (TW).
[c] + = Positive and − = negative reactions in SDS-immunodiffusion tests.

of ZYMV. At least five different virus preparations were analyzed. Yields for ZYMV-TW, -CT, -FL, and -WK averaged 18.8, 14.4, 9.8, and 6.4 mg per 100 g of infected leaf tissue (using an extinction coefficient of 2.4 at A_{260nm} = 1 mg/ml). Virus preparations had a minimal absorption at 247 nm and a maximal absorption at 260 nm. The average A_{260nm}/A_{280nm} ratios were 1.24, 1.22, 1.26, and 1.27 for ZYMV-TW, -CT, -FL, and -WK, respectively.

Serology. The titers of antisera prepared to the four strains of ZYMV were determined in SDS-immunodiffusion tests using homologous virus strains. Different bleeding dates of antisera prepared to ZYMV-CT, -FL, -WK, and -TW had homologous titers up to 32, 32, 8, and 16, respectively (Table 2). Serological relationships between the four ZYMV strains and PMV, BYMV, CYVV, BCMV, PRSV-W, and WMV-2 as determined by SDS-immunodiffusion tests are summarized in Table 2 and Figure 3. Antiserum to ZYMV-CT from early bleedings (3rd–5th) reacted only with WMV-2, PMV, and BCMV, but later bleedings (9th and 10th) also reacted with BYMV and CYVV (Table 2). Antiserum to ZYMV-FL from early bleedings (1st–4th) reacted with WMV-2 and CYVV, and later bleedings (7th–10th) also reacted with PMV and BCMV, but none reacted with BYMV. Antiserum to ZYMV-TW did not react with BYMV or CYVV, but antisera from later bleedings (8th) did react with WMV-2, PMV, and BCMV. Antiserum to ZYMV-WK did not react with WMV-2, BYMV, CYVV, or BCMV, but antiserum from later bleedings (9th) did react with PMV. In reciprocal tests, antisera to WMV-2, PRSV-W, BCMV, CYVV, coat protein, and 54-K nuclear inclusion of PMV failed to react with the four strains of ZYMV (Fig. 3G and I). ZYMV antisera reacted strongly without spurs against antigens of homologous and heterologous strains of ZYMV (Fig. 3A). However, precipitin bands of the ZYMV strains formed definite spur reactions with those formed by WMV-2, PRSV-W, BYMV, CYVV, and BCMV (Fig. 3A–C, E, and F).

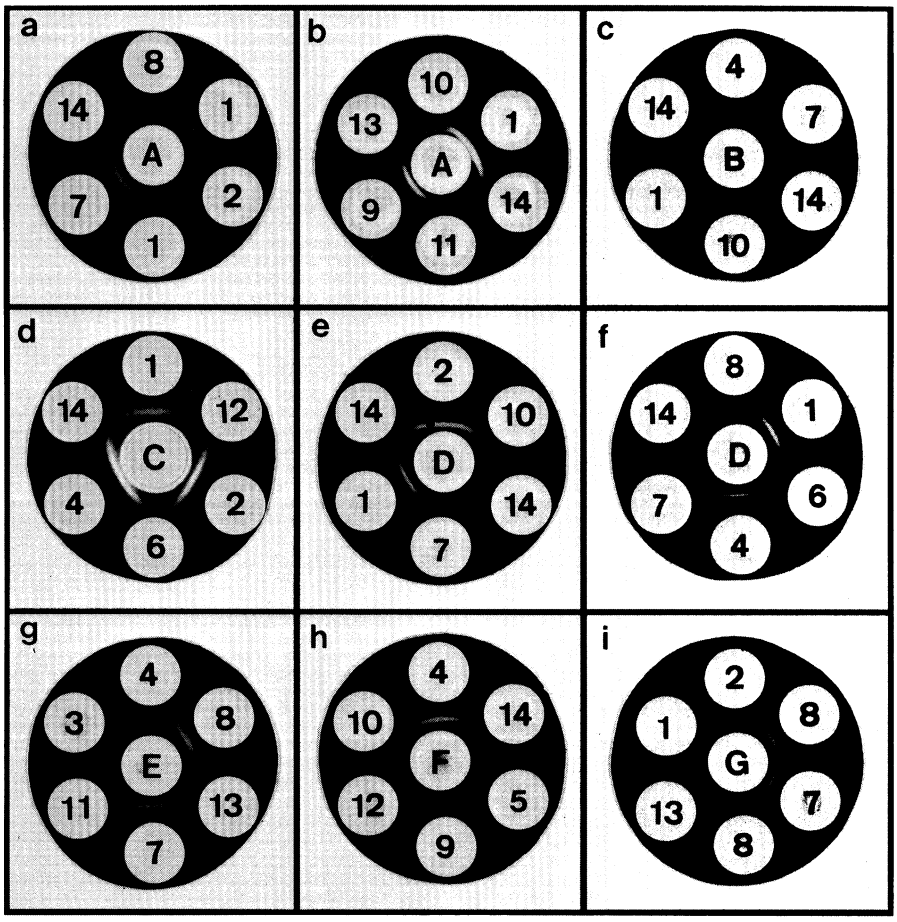

Fig. 3. SDS-immunodiffusion tests with zucchini yellow mosaic virus (ZYMV) strains and other potyviruses. The other potyviruses include: watermelon mosaic virus 2 (WMV-2), pea mosaic virus (PMV), bean yellow mosaic virus (BYMV), bean yellow mosaic virus PV-2 (BYMV PV-2), bean common mosaic virus (BCMV), clover yellow vein virus (CYVV), and papaya ringspot virus watermelon strain (PRSV-W). Center wells contain antisera to **(A and B)** A = ZYMV-CT; **(C)** B = ZYMV-FL; **(D)** C = ZYMV-WK; **(E and F)** D = ZYMV-TW; **(G)** E = PMV coat protein; **(H)** F = ZYMV-CT cytoplasmic inclusion; and **(I)** G = PMV nucleic inclusion. Peripheral wells contained SDS-treated antigens from squash or pea leaves: 1 = ZYMV-CT, 2 = ZYMV-FL, 3 = ZYMV-WK, 4 = ZYMV-TW, 5 = ZYMV-TW, 6 = WMV-2, 7 = PMV, 8 = BYMV PV-2, 9 = BYMV, 10 = BCMV, 11 = CYVV, 12 = PRSV-W, 13 = healthy pea, and 14 = healthy squash.

Noncross-absorbed antibodies of ZYMV-TW reacted to all ZYMV strains in direct DAS-ELISA and indirect

ELISA (Figs. 4A and 5A), although the reactions to heterologous strains were consistently weaker. However, the reactions became specific to ZYMV-TW after cross-absorption with antigens of ZYMV-FL (Figs. 4B and 5B) or ZYMV-CT (Figs. 4C and 5C).

DISCUSSION

Previous reports did not indicate serological heterogeneity of ZYMV strains (13). In SDS-immunodiffusion tests, the four strains of ZYMV we tested appeared indistinguishable (Fig. 3A). However, the relatively weak reaction of ZYMV-TW antibodies with heterologous ZYMV strains in ELISA tests indicated the existence of serological differences between ZYMV-TW and the other three strains (Figs. 4A and 5A). This indication was confirmed by cross-absorption tests (Figs. 4B and C and 5B and C). The results suggest that ZYMV-TW has at least one antigenic determinant that differs from those of ZYMV-FL, -CT, and -WK.

Serological relationships between ZYMV strains and five other potyviruses were demonstrated by SDS-immunodiffusion tests. The results show that ZYMV is related to WMV-2, PMV, BYMV, CYVV, and BCMV (Table 2 and Fig. 3), although detection of these relationships depends on the use of antisera to ZYMV strains and on the antisera collection date after immunization (Table 2). Many reports have indicated that antisera to potyviruses from early bleedings contain virus-specific antibodies, whereas cross-reacting antibodies begin to appear in later stages of bleeding (22,23). The examination of these results have revealed that N- and C-termini of the coat proteins are surface-located and that the N-terminus consists of the only large region in the entire potyvirus coat protein that is virus-specific (22). Because this virus-specific N-terminus is the surface-exposed region in the coat protein of potyvirus, active sites contained in this region should produce virus-specific antibodies in the early stage of immunization. Hence, antibodies to the core protein region of different potyviruses should be produced in the late stages of immunization and are capable of cross-reacting with other potyviruses (6,7,22).

In this study, host range results differed slightly from those of previous reports (11,13,19). Under our conditions, four strains of ZYMV can be distinguished by differences in symptomatology and incubation periods in both squash and melon. However, although the four strains of ZYMV are distinct entities, it is often difficult, under field conditions, for a researcher to differentiate among the symptoms caused by these strains. Therefore, other indicator hosts, Black Turtle #2 and red kidney beans, along with *N. benthamiana*, are useful for differentiation of strains of ZYMV. The cucumber cultivar Taichung Mou Gua was resistant to these four strains (16).

It is known that ZYMV is transmitted by aphids in a nonpersistent manner (9,11,13). In this study, ZYMV-TW had a low transmission rate, even though it reached the highest titer in zucchini 10 days after inoculation. The relatively poor transmission of ZYMV-TW may have been attributable to lower titer in zucchini when the virus source plants were used 6–10 days after inoculation (Fig. 2). The lack of aphid transmissibility for ZYMV-WK could be attributed to the lack of a helper component produced in the plant by the virus (9,11,12).

With potyviruses, particle aggregation causes significant loss of yield during the purification process (24). Our results also show that virus yields are affected by the virus strain and/or the optimal time at which the virus samples are harvested (Fig. 2).

ZYMV is one of the most destructive plant pathogens infecting cucurbit crops in many areas of the world. It has been shown that in squash, it can be effectively controlled in Taiwan (25) and France (10) using the mild strain, ZYMV-WK, characterized in our laboratory. Our ability to differentiate between the protecting ZYMV-WK and the severe ZYMV-TW strain (Figs. 4 and 5) proved valuable in the cross-protection experiment in Taiwan (25). For example, we were able to distinguish severe infections caused by PRSV and ZYMV-TW in cross-protected plants, even though both viruses cause similar symptoms on zucchini. Production of monoclonal antibodies that react only to ZYMV-WK will enable us to study the interaction of mild and severe ZYMV strains in more detail.

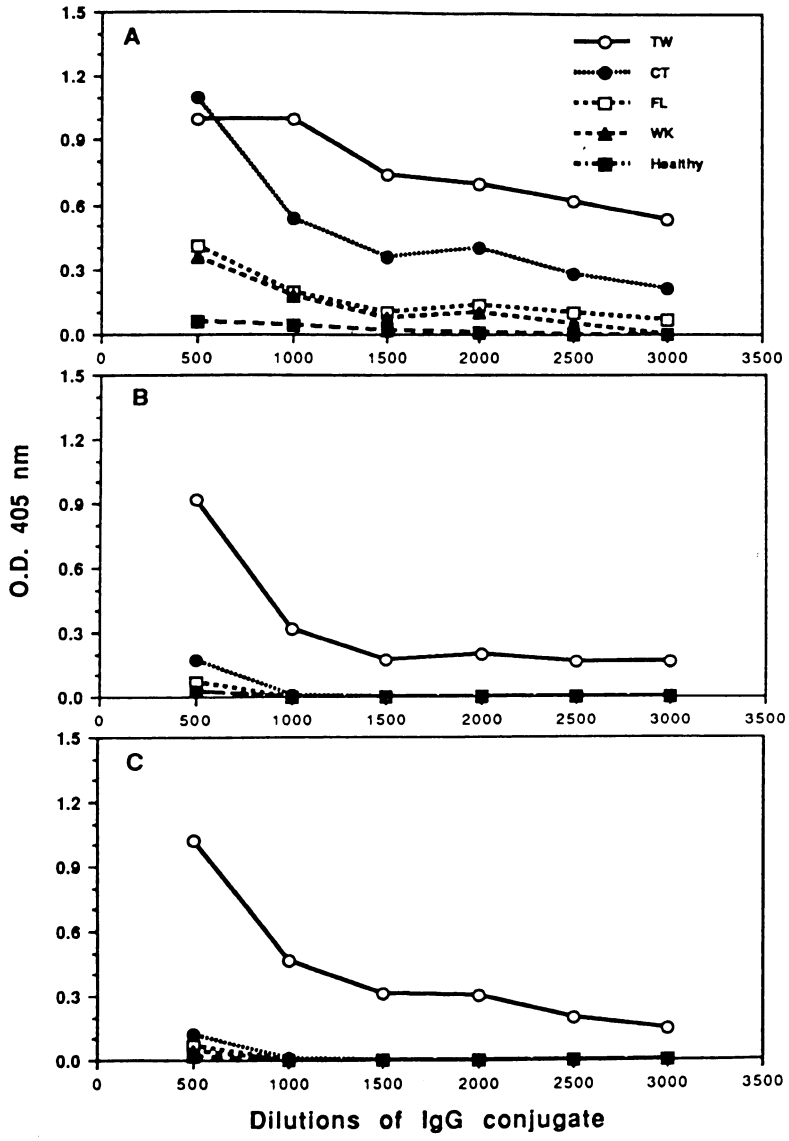

Fig. 4. Serological comparison of four strains of zucchini yellow mosaic virus (ZYMV) in direct ELISA tests. Antiserum to ZYMV-TW was **(A)** not cross-absorbed, **(B)** cross-absorbed with ZYMV-FL, or **(C)** cross-absorbed with ZYMV-CT. The immunoglobulins used were 1.25 μg/ml. The OD_{405nm} values were recorded 30 min after addition of substrate.

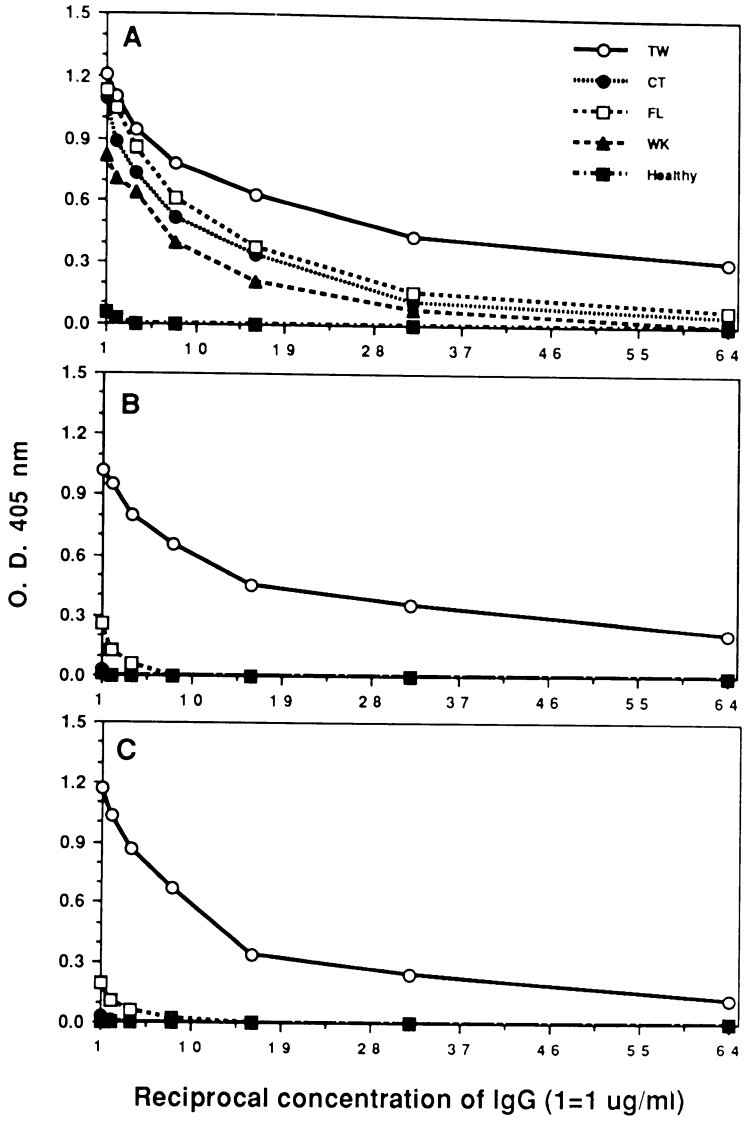

Fig. 5. Serological comparison of four strains of zucchini yellow mosaic virus (ZYMV) in indirect ELISA tests. The antiserum to ZYMV-TW was **(A)** not cross-absorbed, **(B)** cross-absorbed with ZYMV-FL, or **(C)** cross-absorbed with ZYMV-CT. Leaf tissue was triturated in coating buffer at 1:20 dilution (w/v). The OD_{405nm} values were recorded 30 min after addition of substrate.

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