Cytofluorimetric Method for the Detection of the Cucumber Mosaic Virus

D. Iannelli, M. Barba, L. D'Apice, G. Pasquini, R. Capparelli, L. Monti, G. Parrella, F. Scala, and C. Noviello

First, third, and fifth authors: Chair of Immunology, School of Agriculture, University of Naples Federico II; second and fourth authors: Experimental Institute of Plant Pathology, Rome; sixth and seventh authors: Department of Agronomy and Plant Genetics, University of Naples Federico II; and eighth and ninth authors: Institute of Plant Pathology, School of Agriculture, University of Naples Federico II.
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ABSTRACT

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This study describes a method for the detection of cucumber mosaic virus (CMV) by flow cytometry. Extracts from leaves of healthy and virus-infected plants were incubated with latex particles, washed, and then incubated in succession with rabbit anti-CMV antibodies and anti-rabbit immunoglobulin antibodies labeled with fluorescein. Adsorption of CMV virions on the latex particles allowed the virions to become visible to the laser of the cytometer. When the threshold value for virus detection was set at three times that of healthy controls, the detection limit of the cytofluorimetric method was 10 pg/ml of purified virions and that of the enzyme-linked immunosorbent assay (ELISA) was 2.5 ng/ml. The measuring range of the assay described here was 5 ng to 10 pg of purified virions and that of ELISA was 20 to 2.5 ng. The cytofluorimetric method detected the coat protein of CMV in the extract of two transgenic plants at a dilution of 10^{-3}, while ELISA detected the viral protein at a dilution of 5×10^{-2}. The assay was also used to detect plum pox potyvirus and potato virus Y, alone as well as concurrently with CMV.

Additional keyword: dual staining.

Cucumber mosaic virus (CMV) is a positive-strand RNA virus causing severe losses to many cultivated plant species. CMV is distributed worldwide, and its host range exceeds 800 species (20). The virus was first reported in Italy in 1961 (2) and, since then, it has presented a major threat to tomato crops. Many strains of this virus are known (19). These have been classified into two subgroups (I and II) on the basis of their serological characteristics (4), peptide mapping of the viral coat protein (7), and nucleic acid hybridization (12).

The virus can be identified based on host range (21) and serological (23) and molecular tests (22). The present paper describes a new method that is based on flow cytometry. The applications of this technique are becoming increasingly more numerous, since many biological properties can be translated into measurable fluorescence intensity. Flow cytometry is used to measure the DNA content of animal and plant cells (5,9), analyze the cell cycle (1,15), and detect membrane and intracellular antigens (24,28). Applications of flow cytometry to study prokaryotes include microorganism identification (18), cell cycle analysis (17), and assessment of antibiotic resistance (18). In mycology, flow cytometry has been used to measure the DNA content of rust fungi (8).

Flow cytometry allows quantitative measurements at rates of up to 5,000 cells per second (26). However, these measurements can be made only on a population of single cells. To mimic a cell population, CMV virions were extracted from infected plants, adsorbed onto latex particles, and analyzed by flow cytometry.

MATERIALS AND METHODS

Virus isolates. CMV-05, CMV-22, and CMV-PG were isolated in Campania (southern Italy) from commercial fields of tomato plants (14). CMV-05 and CMV-22 belong to subgroup I and CMV-PG to subgroup II.

PPV-ISPAVE 17 was isolated in Piedmont (northern Italy) from a commercial field of apricot trees (cultivar Conte di Costagliole) and multiplied in seedlings of indicator plants (*Prunus persica* (L.) clone GF 305). PVY-Y° was isolated in Emilia (northern Italy) from a commercial field of potatoes and multiplied in *Nicotiana tabacum* L. (cultivar Samsun).

Virion purification and enzyme-linked immunosorbent assay (ELISA) test. The procedures of Lot et al. (16) and Clark and Adams (3) were followed, respectively.

Leaf extracts. Leaves of noninoculated (healthy) plants and CMV-infected plants, inoculated 15- to 30-days postinoculation with 1 µg of purified virions/leaf, were ground in a mortar containing 0.1 M borate buffer (BB) pH 8.5 (1 g of tissue/ml of BB). The extract was centrifuged for 1 min in a microcentrifuge (Eppendorf, Milan), and the supernatant was diluted in BB.

Polyclonal antibodies. The anti-CMV antiserum (anti-CMV) was prepared by immunizing two rabbits with purified virions of isolates CMV-05 and CMV-22. Animals received four subcutaneous injections at 2-week intervals. Each injection consisted of 0.5 mg of virion preparation emulsified with complete Freund's adjuvant. The virion preparation was stabilized with 1% formaldehyde before injection. Fluorescein-labeled antibodies (anti-CMV^{FITC}) were prepared as described by Harlow and Lane (13).

Absorption of anti-CMV. About 5×10^6 latex particles were sensitized overnight at 4°C with 1 ml of extract from a leaf infected with CMV-22 (2 g of tissue/ml of BB). Particles were washed with phosphate-buffered saline (PBS), saturated for 30 min with 1 ml of 3% bovine serum albumin (BSA), and added to 700 µl of anti-CMV diluted to 10^{-3}. The mixture was incubated overnight at 4°C, centrifuged, and the supernatant was then used to test the CMV-PG isolate.

Monoclonal antibodies. Two BALB/c mice were immunized with purified and formaldehyde-stabilized virions (isolate CMV-05). Animals received two intraperitoneal injections at 3-week

Corresponding author: C. Noviello; E-mail address: scala@unina.it

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intervals. Each injection consisted of 50 to 80 μg of purified virions emulsified with complete Freund's adjuvant. Spleen cells were fused to NS1-Ag18 myeloma cells by the method of Galfré and Milstein (10). The unlabeled antibodies were designated as A3H1 (from the name of the clone) and the fluorescein-labeled antibodies (13) as A3H1^{FITC}.

The monoclonals specific for plum pox potyvirus (PPV) (clone B7C4) and for potato virus Y (PVY) (clone 2D) were prepared essentially by the procedure outlined above. The isolates used for immunization of mice were PPV-ISPAVE 17 and PVY-Y°.

Standard cytofluorimetric test (SCT). About 10^7 latex particles (Polysciences, Ltd., Eppelheim, Germany) were incubated (overnight at 4°C or 2 h at room temperature) under agitation with 1 ml of leaf extract. All other steps were carried out at room temperature. The size of the particles (1, 3, or 6 μ) and the number of particles per tube (10^6, 2.5×10^6, or 5×10^6) were as indicated in each experiment. The mixture was centrifuged, and the pellet was incubated for 30 min with 1% BSA in BB. The latex particles were then washed with 0.15 M PBS (pH 7.2), incubated for 4 h with primary antibodies diluted in PBS (rabbit polyclonal antibodies for CMV and monoclonal antibodies for PPV or PVY), washed with PBS, and incubated for 1 h with secondary antibodies. These were goat anti-rabbit immunoglobulin-labeled with fluorescein (anti-R^{FITC}) for CMV and goat anti-mouse immunoglobulin-labeled with fluorescein (anti-M^{FITC}) for PPV or PVY. Anti-R^{FITC} and anti-M^{FITC} reagents were purchased from Sigma Chemical Co. (St. Louis). Particles were washed once with PBS and tested with the flow cytometer. Controls of extract samples were incubated with PBS instead of primary antibodies. The instrument (FACScan; Becton Dickinson Immunocytometry Systems, San Jose, CA) was equipped with a 15-mW, air-cooled, 488-nm argon ion laser. Green fluorescence (fluorescein isothiocyanate, FITC) was collected through a 530/30-nm band pass filter. The data of 10,000 events were collected for each sample, stored in list mode, and analyzed using Consort 32 software (Hewlett-Packard Co., Sunnyvale, CA). Forward (FSC) and side (SSC) scattering were analyzed on a linear scale and FITC fluorescence on a logarithmic scale. FSC measured the size and SSC the granularity (fine internal structure) of the particles. No gates were set around the particles. Results are presented as the mean channel of fluorescence of the treated sample subtracted by the mean channel of the control. Logarithmic units ($\log_{10}$ U) were transformed into linear channels (LC) by the formula: LC = total number of channels/number of log decades × $\log_{10}$ U. The total

TABLE 1. Detection of cucumber mosaic virus (CMV) by the standard cytofluorimetric test

	Relative change in sample fluorescence intensity[a]							
	5×10^{-1}[b]		10^{-1}[b]		10^{-2}[b]		10^{-3}[b]	
Anti-CMV dilution	I[c]	H[d]	I	H	I	H	I	H
10^{-3}	299 ± 1.4	171 ± 2.4	369 ± 1.4	281 ± 4.9	181 ± 1.4	160 ± 2.4	171 ± 2.4	167 ± 3.5
10^{-4}	160 ± 2.4	19 ± 3	235 ± 0.8	0	95 ± 0.7	0	65 ± 2.1	0
5×10^{-5}	45 ± 1.3	0	140 ± 1.4	3 ± 0.12	45 ± 1.3	0	25 ± 0.7	0

[a] Values (mean channel of fluorescence of the treated sample subtracted by the mean channel of the control) are the average of three experiments ± the standard deviation. The size of the particles was 3 μ and the number of particles per tube was 5×10^6.
[b] Extract dilution.
[c] I = infected; i.e., extract from leaves infected with CMV (isolate CMV-22).
[d] H = healthy; i.e., extract from control (noninoculated) leaves.

TABLE 2. Influence of the particle number on the sensitivity of the standard cytofluorimetric test

	Relative change in sample fluorescence intensity[a]							
	5×10^{-1}[b]		10^{-1}[b]		10^{-2}[b]		10^{-3}[b]	
Number of particles per tube (10^6)	I[c]	H[d]	I	H	I	H	I	H
5	160 ± 2.4	19 ± 3	235 ± 0.8	0	95 ± 0.7	0	65 ± 2.1	0
2.5	171 ± 2.4	9 ± 2.8	261 ± 4.9	0	184 ± 0.7	0	140 ± 1.4	0
1	181 ± 1.4	25 ± 0.7	328 ± 2.8	3 ± 0.32	235 ± 0.8	0	140 ± 1.4	0

[a] Values (mean channel of fluorescence of the treated sample subtracted by the mean channel of the control) are the average of three experiments ± the standard deviation. The size of the particles was 3 μ and the dilution of anti-CMV was 10^{-4}.
[b] Extract dilution.
[c] I = infected; i.e., extract from leaves infected with CMV (isolate CMV-22).
[d] H = healthy; i.e., extract from control (noninoculated) leaves.

TABLE 3. Specificity of the standard cytofluorimetric test

	Relative change in sample fluorescence intensity[a]				
Virus	10^{-1}[b]	10^{-2}[b]	10^{-3}[b]	10^{-4}[b]	10^{-5}[b]
RMV[c]	19 ± 3	0	0	ND[d]	ND
TAV[e]	45 ± 1.3	38 ± 2.8	3 ± 0.5	ND	ND
CMV-22	328 ± 2.8	263 ± 5	158 ± 2.4	79 ± 0.7	65 ± 2.1
CMV-05	299 ± 1.4	211 ± 2.6	95 ± 2	57 ± 2.1	25 ± 0.7
CMV-PG	25 ± 0.7	3 ± 0.2	0	ND	ND
H[f]	3 ± 0.5	0	0	0	0

[a] Values (mean channel of fluorescence of the treated sample subtracted by the mean channel of the control) are the average of two experiments ± the standard deviation. The size of the particles was 3 μ, the dilution of anti-CMV was 10^{-4}, and the number of particles per tube was 10^6.
[b] Extract dilution of the extract from leaves infected with the virus indicated in the table.
[c] Robinia mosaic virus.
[d] ND = not determined.
[e] Tomato aspermy virus.
[f] H = healthy; i.e., extract from control (noninoculated) leaves.

number of channels and the number of log decades of the instrument were 1,024 and 4, respectively.

Modified cytofluorimetric test (MCT). The assay was carried out at room temperature. Latex particles (1, 3, or 6 μ) were coated (2 h) with either anti-CMV- or A3H1-purified antibodies (11) (1 ml at 100, 10, or 1 μg/ml and 10^7 particles), quenched with 1% BSA for 30 min, and incubated for 2 h with 1 ml of the extract to be tested. The latex particles were then washed again and incubated with the fluorescein-labeled reagent. This was anti-CMV^{FITC} (diluted to 2×10^{-3}) for particles coated with A3H1 and A3H1^{FITC} (diluted to 2×10^{-2}) for particles coated with anti-CMV.

Dual cytofluorimetric test (DCT). Latex particles were incubated with 1:1 (vol/vol) pool of leaf extracts from plants infected separately with CMV and PVY or separately with CMV and PPV. Latex particles were then treated with the corresponding primary antibodies (same as in SCT) and with two secondary antibodies (phycoerythrin-conjugated anti-rabbit and fluorescein-conjugated anti-mouse, both diluted to 4×10^{-3} with PBS). The rest of the assay was as described for SCT.

RESULTS

Set up and optimization of SCT for CMV detection. In a preliminary experiment, different dilutions of extracts from leaves infected with isolate CMV-22 and a control (noninoculated) plant were incubated overnight with a constant number of latex particles

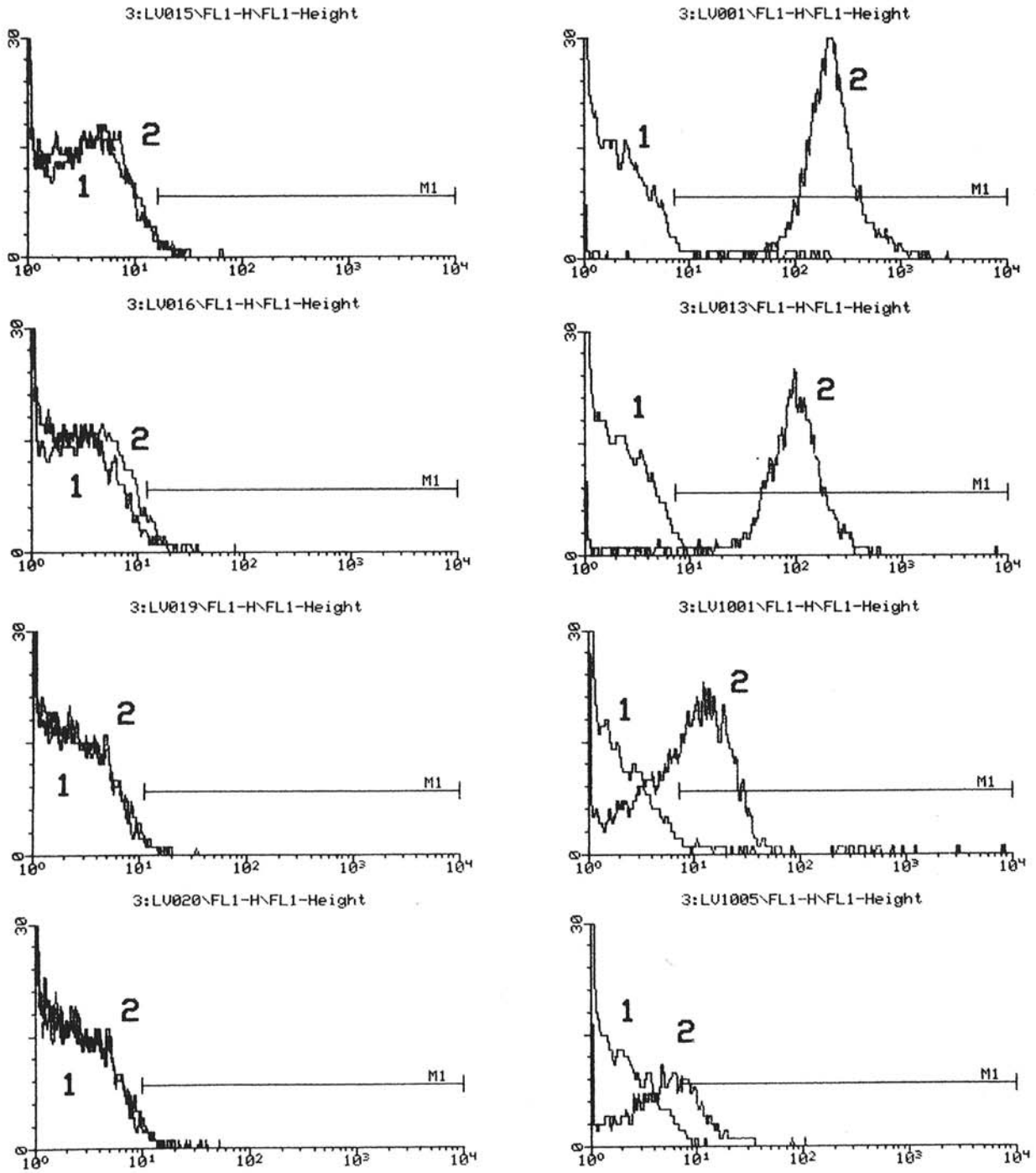

Fig. 1. Cytofluorimetric profile of four different dilutions (from top to bottom: 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of extracts from leaves of a healthy plant (left) and from an infected plant (right). Abscissa: relative change in sample fluorescence intensity. Ordinate: number of particles. Extracts tested without (curve 1) and with (curve 2) anti-cucumber mosaic virus (CMV). In the case of the extract from the infected plant, the relative change in sample fluorescence intensity (from top to bottom) was 454, 346, 210, and 65.

(about 5×10^6 3-μ particles/tube) and then tested with different dilutions of anti-CMV. The anti-R^{FITC} was used in excess (at a dilution of 3×10^{-3}). The results suggested that the two extracts could be differentiated. The difference was particularly evident when anti-CMV was diluted to 10^{-4} and extracts to 10^{-1} (Table 1).

In a second experiment, using anti-CMV diluted to 10^{-4} and 5×10^{-5} (the dilutions that in the previous experiment were more discriminating), the number of latex particles per tube was varied. This experiment showed that by reducing the particle number, the sensitivity of the test could be improved. The results observed with the 10^{-4} dilution of the anti-CMV are shown in Table 2.

The influence of the particle size on the sensitivity of the assay was examined next. The 3-μ particles gave better results than the 6- and 1-μ particles. Finally, we optimized the incubation time of particles with extracts and the dilution of anti-R^{FITC}. The incubation of particles with extracts could be shortened to 2 h at room temperature (instead of overnight) and the anti-R^{FITC} could be diluted to 10^{-3} (instead of 3×10^{-3}) without influencing the outcome of the test (data not shown). Under the conditions defined by the experiments described above, infected and healthy plants were still distinguishable when extracts were diluted to 10^{-4} or further (Fig. 1).

Specificity of SCT. The specificity of the method was assessed by testing extracts from plants experimentally infected with viruses serologically related to CMV (robinia mosaic virus and tomato aspermy virus) with the subgroup I isolates CMV-22 and CMV-05 and the subgroup II isolate CMV-PG. When the antiserum was diluted to 10^{-4}, antibodies bound significantly only to the homologous isolates (Table 3). When the antiserum was diluted to 10^{-3} and absorbed with the extract of a plant infected with CMV-22, the antibodies were able to recognize the CMV-PG isolate also; in two experiments the average fluorescence intensity was 369 ± 1.4. In ELISA, the same antiserum, nonabsorbed and diluted to 10^{-3}, reacted with both subgroup I and subgroup II isolates (data not shown).

Sensitivity and measuring range of SCT. Purified CMV-22 virions were used as standards to evaluate the sensitivity and range of measurement of SCT and ELISA. The mean channel of fluorescence of extracts from healthy plants diluted to 10^{-1} or more varied between 3 and 0 (Table 3). The absorbance of the same extracts varied between 5 and 7%. When the threshold value for virus detection was set at three times that of healthy controls (22), the detection limit of SCT was 10 pg/ml and of ELISA was 2.5 ng/ml. The measuring range of SCT (given by the linear portion of the standard curve) was 5 ng to 10 pg and that of ELISA was 20 to 2.5 ng (Fig. 2). When the purified virions were diluted in extracts from a noninoculated plant diluted to 10^{-1}, instead of BB, the results were similar to those reported in Figure 2. Both the intra- and interassay coefficients of variation (CV) of SCT were below 5% at all concentrations higher than 10 pg/ml. The intra- and interassay CV of ELISA were 10 and 14%, respectively, at all concentrations higher than 3 ng/ml. Thus, SCT is a highly sensitive immunoassay with a wide measuring range.

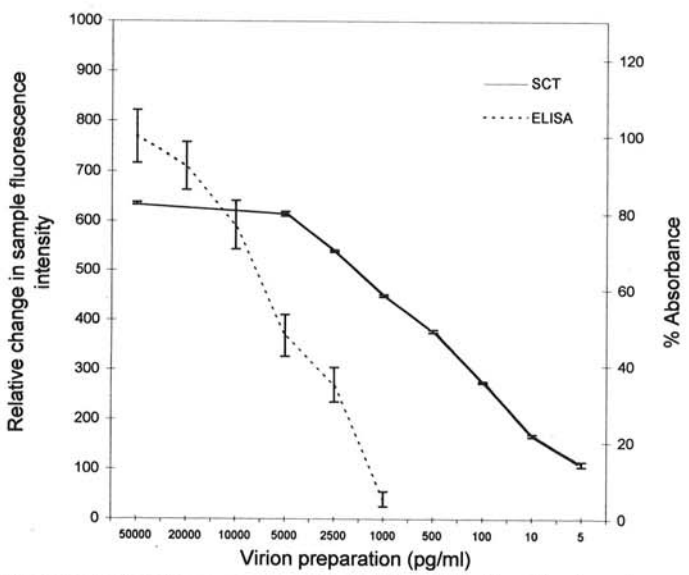

Fig. 2. Detection of cucumber mosaic virus by standard cytofluorimetric test (SCT) and enzyme-linked immunosorbent assay (ELISA). Comparison of sensitivity and measuring range of the two methods. Bars indicate the standard deviation. The threshold value for virus detection was three times the value of the healthy control: $3 \times 51 \pm 2.3$ for SCT and $3 \times 7 \pm 0.98$ for ELISA.

Detection of the virus coat protein in transgenic plants. Extracts of leaves from two transgenic tomato plants expressing subgroup I CMV coat protein (25) were tested in parallel by SCT and ELISA. The former test detected the viral protein at a dilution of 10^{-3} of the extract and the latter at a dilution of 5×10^{-2} (data not shown).

Comparison of SCT and MCT for virus detection. The aim of this experiment was to ascertain whether SCT could be further improved by incubating latex particles first with antibodies specific to CMV and then with the plant extract. This test is referred to as MCT. Different dilutions of the same extract from CMV-05-infected leaves were incubated with a constant number of uncoated or antibody-coated (anti-CMV or A3H1) latex particles, washed, and incubated again with the fluorescein-labeled reagent (Materials and Methods). Antibody-coated particles upon incubation with the virion preparation tended to aggregate, as demonstrated by the increased FSC of the particles (Fig. 3). FSC measured the size of the objects (in this case, latex particles) illuminated by the laser. Contrary to expectation, antibody-coated particles gave a weaker fluorescent signal (Table 4). Varying the number of particles per tube (10^6 to 2.5×10^6 or 5×10^6), the size of the particles (1, 3, or 6 μ), or the coating antibody (anti-CMV or A3H1) did not improve the performance of the test (data not shown).

Detection of PPV by SCT. The high sensitivity displayed by SCT in the detection of CMV prompted us to apply the method for the detection of PPV, known to be difficult to identify by conventional procedures. Extracts from infected and healthy leaves of the indicator plant diluted to 10^{-1} were clearly distinguishable (data not shown). When the threshold value for PPV detection was set at three times that of the healthy control, as done for CMV, the sensitivity of the test was 500 pg of virus/ml and the measuring range was 250 ng to 500 pg (Fig. 4).

Simultaneous detection of CMV and PVY. The potential of the cytometer to carry out two immunofluorescent measurements at the same time was exploited (Materials and Methods) to simultaneously detect two distinct viruses. Double-color immunofluorescence analysis revealed that these two viruses could be identified simultaneously. The two immunofluorescence signals did not influence each other at all. The experiments were repeated with CMV and PPV. Again, the two viruses could be identified concurrently and without interference between fluorochromes (Table 5).

DISCUSSION

CMV is easily detected by the traditional and modern methods already available. An additional test for CMV would, therefore, not seem particularly useful, unless it contains novel potentialities in comparison with those already existing. We believe this paper demonstrates for the first time that a plant virus can be detected by cytofluorimetry. One justification for describing this method is that it represents a model that can be applied to viruses that are more difficult to identify than CMV, such as the closteroviruses associated with grapevine leafroll (GLRaV), prune dwarf ilarvirus (PDV), and PPV. This claim is supported by the evidence pro-

vided in the present paper that the assay can be easily adapted to detect PPV also.

Using antibodies labeled with different fluorochromes, cytofluorimetry allows the simultaneous identification of two distinct viruses. This represents another positive feature of SCT, in view of the fact that detection of two viruses occurs in the absence of synergistic or antagonistic reactions between fluorochromes. The intensity of the signals (the mean channel values) did not change

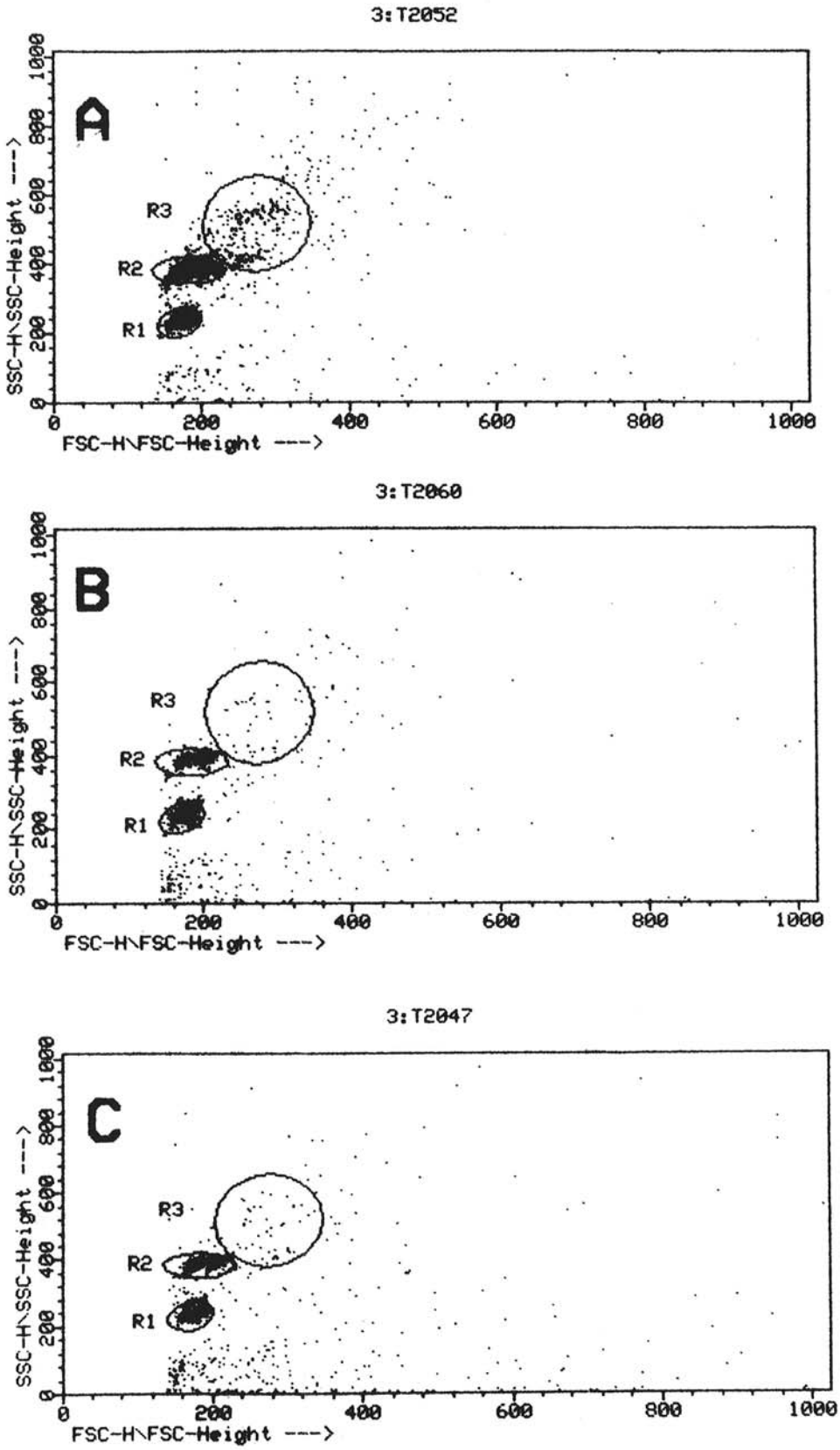

Fig. 3. Dot plot showing forward-light scatter (abscissa) versus side-light scatter (ordinate) of latex particles **A,** incubated with anti-cucumber mosaic virus (CMV) and then with the virus; **B,** incubated directly with the virus; and **C,** incubated with neither the anti-CMV nor the virus. Side-scatter heterogeneity (regions R1 and R2) is an intrinsic property of the latex particles and **C,** is also present in the untreated particles. Upon incubation with the virus, antibody-coated latex particles tend to aggregate, as displayed by the higher number of particles with increased forward scatter (region R3).

TABLE 4. Comparison of the standard cytofluorimetric test (SCT) with the modified cytofluorimetric test (MCT)

	Relative change in sample fluorescence intensity[a]			
	10^{-2}[b]	10^{-3}[b]	10^{-4}[b]	10^{-5}[b]
SCT	523 ± 0.7	523 ± 0.7	210 ± 2.4	79 ± 0.7
MCT	210 ± 2.4	171 ± 2.4	80 ± 0.7	65 ± 2.1

[a] Values (mean channel of fluorescence of the treated sample subtracted by the mean channel of the control) are the average of two experiments ± the standard deviation. The size of the particles (3 μ) and the number of particles per tube (10^6) were the same in both assays. The fluorescence of the extract from healthy leaves was 0 in both assays.

[b] Extract dilution of the extract from leaves infected with cucumber mosaic virus (CMV) (isolate CMV-22).

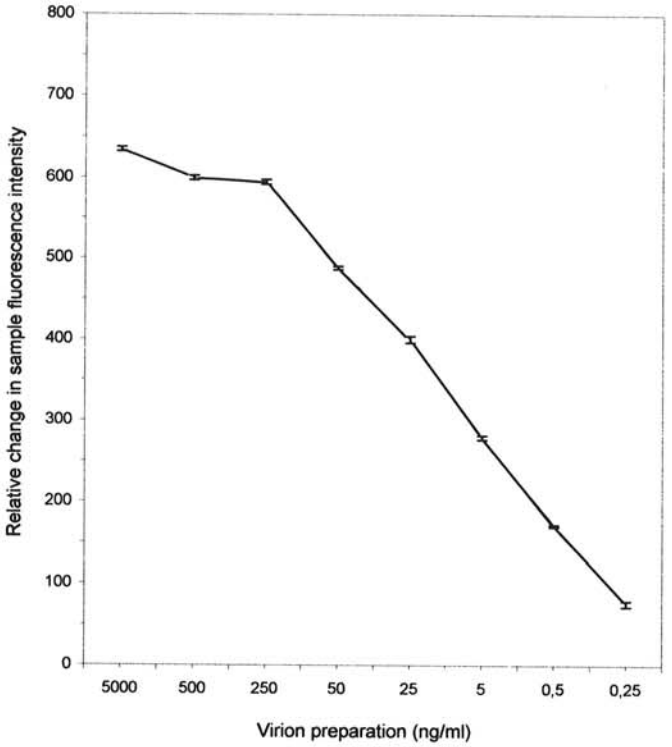

Fig. 4. Detection of plum pox potyvirus by standard fluorometric test. Sensitivity and measuring range of the test. Bars indicate the standard deviation. The threshold value for virus detection was three times the value of the healthy control: 3 × 46 ± 1.4.

when PPV and PVY were detected alone or in association with CMV (Table 5).

SCT can detect the virus coat protein in the extract of transgenic plants at a dilution of 10^{-3}. ELISA can detect the protein in the same extract at a dilution of 5×10^{-2}. In addition, as discussed above, SCT has the potential to ascertain the presence of the coat protein of two distinct viruses at the same time.

ELISA is the test most frequently used for the identification of CMV, but it is not without pitfalls, such as the occurrence of false negatives and a too high background of virus-free controls (21). High inter- and intraassay CV often represent another limit of the technique. SCT compares favorably for sensitivity with ELISA (Fig. 2) and, in the case of CMV, also with PCR (Table 6).

CV as low as 5% can be attained with SCT. This is possible because the method permits the use of unchanged gate and marker settings throughout the experiments, i.e., optimized and uniform testing conditions for all samples. The method also requires short incubation times. More than 50 samples (and probably much more) can be tested in 1 working day. Thus, the test has the essential requirements for routine work. At present, its main limitation is the high cost of the cytometer. However, this instrument, in

TABLE 5. Simultaneous detection of two virions by the standard cytofluorimetric test

Relative change in sample fluorescence intensity[a]					
Experiment 1			Experiment 2		
Virion	FITC[b]	PE[c]	Virion	FITC	PE
CMV-22	...	503 ± 4	CMV-22	...	523 ± 4
PVY[d]	65 ± 2.1	...	PPV[e]	79 ± 0.7	...
CMV-22 + PVY	65 ± 2.1	503 ± 4	CMV-22 + PPV	79 ± 0.7	523 ± 4

[a] Values (mean channel of fluorescence of the treated sample subtracted by the mean channel of the control) are the average of two experiments ± the standard deviation. The size of the particles was 3 μ, the dilution of anti-CMV was 10^{-4}, and the dilutions of anti-PVY and anti-PPV monoclonals were 2×10^{-3}.

[b] Fluorochrome of fluorescein isothiocyanate (FITC).

[c] Fluorochrome of phycoerythrin (PE).

[d] Potato virus Y.

[e] Plum pox potyvirus.

TABLE 6. Sensitivity (pg of purified virion/ml) of enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and the standard cytofluorimetric test (SCT) in the detection of cucumber mosaic virus (CMV) and plum pox potyvirus (PPV)

	Sensitivity	
Assay	CMV	PPV
ELISA	1,000[a] to 2,500[b]	4,000[c]
PCR	10[a]	200[c]
SCT	10[b]	500[b]

[a] From Ilardi et al. (14).

[b] From this paper.

[c] From Wetzel et al. (27).

view of its versatility, is expected soon to become widely used in many fields of plant biology (9). The availability of visible diode lasers of low price, high efficiency, and long lifetime (6) is expected to promote broader application of flow cytometry.

Subgroup I and subgroup II CMV isolates were tested by SCT and ELISA. The same rabbit antiserum was used in both assays. The optimal dilution of the antiserum was 10^{-3} for ELISA and 10^{-4} for SCT. In the latter assay, the 10^{-3} dilution displayed a reaction also with the extract from healthy plants and, thus, could not be used (Table 1). Under the above conditions, SCT was subgroup I-specific (Table 3), while ELISA reacted with the isolates of both subgroups. The explanation proposed to account for the difference in specificity of the two methods is that the titer of the antibodies specific for subgroup I isolates is higher than that of the antibodies recognizing subgroup II isolates. In particular, at a 10^{-4} dilution, only subgroup I-specific antibodies are capable of reacting with the virions. When subgroup I-specific antibodies are removed by absorption, the antiserum can be used in SCT diluted to 10^{-3}, and the presence of subgroup II antibodies becomes evident. The absorption results support this explanation and, at the same time, demonstrate how the specificity of an antiserum may be influenced by the sensitivity of the assay employed.

Finally, the lower sensitivity of MCT in comparison with SCT deserves a comment. A plausible explanation for both the aggregation of the latex particles and the weaker signal observed with the MCT could be the following: the antibodies, being much smaller than the CMV virions, may adsorb onto the latex beads at high density creating a favorable condition for several beads to interact with a single virion and to aggregate. Aggregation of the beads, in turn, impedes the free access of the second antibody (the fluorescent one) to the virus particles, and the signal becomes consequently weaker.

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