Cucumber Mosaic Cucumovirus Populations in Italy Under Natural Epidemic Conditions and After a Satellite-Mediated Protection Test

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ABSTRACT

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More than 2,000 samples of weeds and cultivated crops were collected in Italy from areas where cucumber mosaic cucumovirus (CMV) epidemics occurred in 1988 and 1989. A collection of weeds was also made from fields neighboring the site of a 1989 satellite-mediated protection test of tomato. Samples were screened by molecular hybridization analyses, and CMV isolates were typed on the basis of their ability to hybridize with subgroup-specific riboprobes and with a satellite RNA-specific riboprobe. A strong relationship between CMV strain and disease type was found in tomato. The etiology of the so-called lethal necrosis syndrome was related to a helper virus belonging to the S subgroup, and a CMV strain assigned to the WT subgroup was involved in the fruit necrosis syndrome. Virus strains belonging to the WT subgroup were more widely distributed on different plant species and therefore present throughout the year, whereas those of S-type occurred more frequently from winter to early spring. Samples from weeds, celery, and melon were collected around the site of the 1989 satellite-mediated protection test. The protective strain used in that study (CMV-S) belongs to the S subgroup, and no increase in the S subgroup CMV population was observed. Specific CARNA 5 sequences were recognized either in cultivated crops or in weeds even when the helper virus was no longer detectable. A seemingly new CARNA 5 variant named Tfn-CARNA 5 was found associated with tomato fruit necrosis.

Cucumber mosaic cucumovirus (CMV) has small icosahedral virions with three plus-sense genomic RNAs and a fourth subgenomic RNA, which acts as the messenger RNA for coat protein synthesis (for review see 14). Some CMV strains often support the replication of a fifth small (330–370 nucleotides) linear satellite RNA (3,15), here designated CARNA 5 (for CMV-associated RNA 5), which is capable of modulating plant disease symptoms induced by the helper virus (7). The virus induces severe diseases in a wide range of herbaceous and woody plants, and the epidemiology of such diseases is often obscured by the ability of the virus to survive in weed hosts and to be transmitted by more than 60 species of aphids in a nonpersistent manner (10).

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In 1988 and 1989, massive CMV outbreaks occurred in vegetable crops of four Italian regions: Apulia, Basilicata, Campania, and Emilia-Romagna. Canning tomatoes were most affected, but the epidemic also involved pepper, melon, watermelon, cucumber, zucchini squash, and celery (1,4). Three different syndromes were observed in tomato: typical fernleaf-shoestring (10) (mainly in Apulia and Campania); lethal necrosis or tomato necrosis (9) (mainly in Basilicata and Emilia-Romagna), and fruit necrosis (mainly in Campania and sporadically in Apulia). Fruit necrosis is characterized by the presence of a variously extended internal browning of fruits borne by vigorous plants showing no symptoms, or at most a mild mottling of the leaves. Lethal necrosis and fruit necrosis are new to Italian tomato crops. Whereas lethal necrosis was related to a CMV strain (CMV-PG) carrying a necrogenic variant of its satellite RNA (designated PG-CARNA 5) (8), fruit necrosis was induced by a CMV strain supporting a nonnecrogenic variant of CARNA 5 (A. Crescenzi and D. Gallitelli, *unpublished*).

As an attempt to control lethal necrosis epidemics, a test was carried out in Basilicata in 1989 in which tomato plants were preventively inoculated with a mild strain of CMV (CMV-S) carrying a nonnecrogenic CARNA 5 variant (S-CARNA 5) and exposed to natural infections. The results were very encouraging (5), but questions were raised about the safety of the test regarding the possible dissemination of the satellite RNA used for cross-protection.

In order to identify CMV strains present in naturally infected plants after the epidemics, and to obtain a preliminary assessment of the safety of the satellite-mediated protection tests (SPT), samples from weeds and cultivated plants were collected for 2 yr. CMV typing was done with molecular probes that specifically recognize isolates of the two subgroups of CMV identified by genomic sequence homology (16,17), which also correspond to the serogroups designated by Devergne et al (2). The results of this survey are the subject of the present paper.

MATERIALS AND METHODS

Collection of field samples. In Apulia, Basilicata, and Campania, samples were collected in 1989 and 1990, but in Emilia-Romagna samples were collected only in 1989. Crop plants (mainly tomato and celery) from commercial fields were sampled throughout the vegetative season. Weeds were sampled in Basilicata and Campania from January to June, before and after tomato seedlings were transplanted in the field. In particular, five sampling areas for weed species were selected in Basilicata, three of which border the place (Cobas) where lethal necrosis had developed and the SPT was

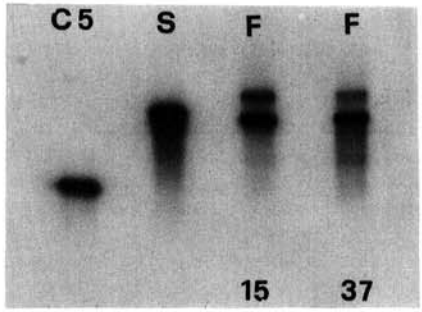

Fig. 1. Agarose gel electrophoresis of minus-sense RNA transcripts obtained from clones of D-CARNA 5 (C5), CMV-S (S), and CMV-Fny (F). Transcription from the Fny clone was carried out either at 37 C or 15 C.

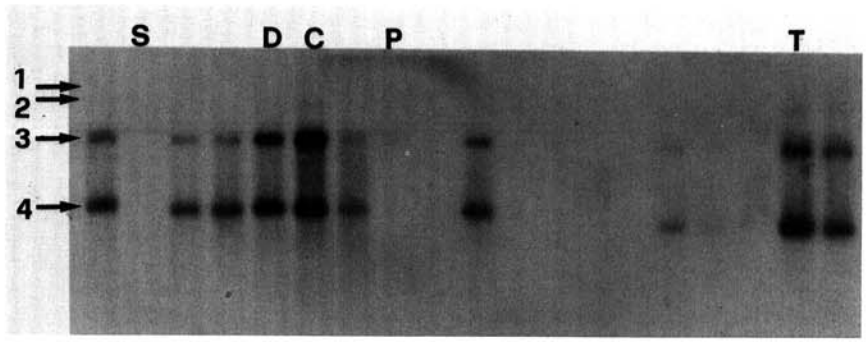

Fig. 2. Northern blot and hybridization with Fny riboprobe of CMV RNAs extracted from control strains and from some isolates purified from naturally infected tissues. S = CMV-S, D = CMV-D, C = CMV-CR (purified from celery), P = CMV-PG (purified from tomato), and T = CMV-Tfn (purified from tomato). The probe hybridizes poorly with viral genomic RNAs 1 and 2.

done. In Campania, weed samples were collected mainly in the proximity of commercial tomato fields with a high incidence of fruit necrosis. Whenever possible, the same weed species were collected from the same place repeatedly from January to June.

The CMV strains CMV-1, CMV-D, and CMV-S were maintained in Rutgers tomato seedlings and used as controls.

Virus purification and nucleic acid extraction. Tissues from naturally infected tomato plants were used either for CMV purification according to the procedure of Lot et al (13) or for mechanical inoculation of Rutgers tomato seedlings at the cotyledonary stage. Purified virus particles were suspended in 50 mM NaCl and stored at −20 C with 30% glycerol or used immediately for nucleic acid extraction. To extract viral RNA, CMV particles were incubated at room temperature in Tris-EDTA (TE) buffer (20) containing 0.1% sodium dodecyl sulfate and extracted twice with TE-saturated phenol-chloroform (1:1, v/v) (chloroform was chloroform-isoamyl alchohol, 24:1, v/v). After two ethanol precipitations in the presence of 200 mM sodium acetate (pH 6), viral nucleic acid was suspended either in TE or RNAse-free distilled water, and the final concentration was adjusted to 1 mg/ml. When only a few grams of plant tissue was available (mainly with weeds), the method of White and Kaper (25) for total nucleic acid extraction was adopted. Total nucleic acid (TNA) extracts were suspended in Tris-acetate-EDTA buffer (20) containing 8 M urea and 0.3% bromophenol blue and stored at −70 C.

Viral nucleic acid analysis. Viral nucleic acid (0.5–1 mg) extracted from purified particles was analyzed by gel electrophoresis in 1.2% agarose in Tris-borate-EDTA (20) after partial denaturation with 80% deionized formamide at 85 C for 2 min. When necessary, after electrophoresis and ethidium bromide staining, RNA bands were directly transferred to nylon membranes (Amersham Hybond N) by capillary blotting. TNA extracts were analyzed by polyacrylamide gel electrophoresis (PAGE) under semidenaturing conditions (25). After electrophoresis, nucleic acid bands were stained, subjected to RNAse treatment, and blotted onto nylon membranes as described by White and Kaper (25). For dectection by dot hybridization, 25 μl of TNA extracts was loaded into each well of a filtration apparatus (American Bionetics) connected with a vacuum line. Nucleic acids were fixed to nylon filters by 5-min exposure to ultraviolet light.

In vitro transcription and molecular hybridization analysis. Minus-sense RNA in vitro transcripts from the following recombinant plasmids were used as probes: RNA 3 (2.03-kb insert) from CMV-Fny cloned in pBS M13− (provided by P. Palukaitis, Cornell University, Ithaca, New York); RNA 3 (1.4-kb insert) from CMV-S cloned in pGEM3Zf+ (provided by F. Cellini, Metapontum Agrobios, Metaponto, Italy); and D-CARNA 5 (0.335-kb insert) from CMV-D cloned in pSP65 (provided by J. M. Kaper, U.S. Department of Agriculture, Beltsville, Maryland). To synthesize minus-sense RNA transcripts, recombinant plasmid DNA templates obtained by the boiling method (20) were linearized with *Bam*HI (for CMV-Fny and CMV-S) or with *Hin*dIII (for D-CARNA 5). In vitro transcription of the CMV-Fny and CMV-S plasmids were carried out with T7 RNA polymerase (Boehringer Mannheim), and transcription of the D-CARNA 5 plasmid was done with SP6 RNA polymerase (Bethesda Research Laboratory). Transcription was carried out as recommended by the respective manufacturers, using [^{32}P]uridine 5′-triphosphate (800 Ci/mM) (Amersham) as a radioactive label. RNA transcripts were separated from unincorporated nucleotides with Bio Spin 30 chromatography columns (Bio-Rad Laboratories). Filters were prehybridized for 2 hr and hybridized for 16 hr either at 63 C (high stringency) or 42 C (low stringency) in 50% formamide/5× SSPE (saline-sodium phosphate-EDTA; 20× SSPE is 3.6 M NaCl, 200 mM sodium phosphate, pH 7.7, 2 mM EDTA) containing 5× Denhardt's solution and 240 mg/ml single-stranded sonicated DNA from salmon sperm (20). The hybridization mixture contained 1×10^6 cpm/ml of the synthesized transcript. After hybridization, filters were washed four times for 30 min at 65 C in 0.1× SSC (saline-sodium citrate; 20× SSC is 3 M NaCl, 300 mM sodium citrate) and then exposed at −70 C with intensifying screens against Fuji X-ray films. When necessary, hybridized material was removed by boiling the filters twice for 15 min in distilled water containing 0.1% sodium dodecyl sulfate.

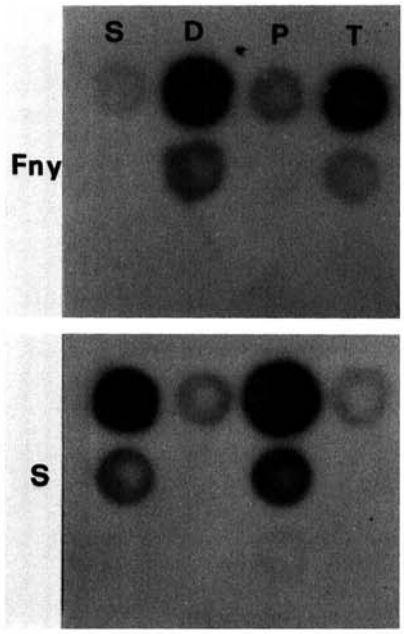

Fig. 3. Dot blot hybridization under high stringency of viral RNAs extracted from purified particles of CMV-S (S), CMV-D (D), CMV-PG (P), and CMV-Tfn (T). The two riboprobes (Fny and S) clearly distinguish CMV strains belonging to different subgroups. From top to bottom, the three spots of each CMV strain contain 12, 1.2, and 0.12 ng of viral RNA.

RESULTS

Optimization of hybridization tests. In vitro transcription produced full-length,

minus-sense transcripts for all clones used. However, the presence of some less than full-length (about 1.4 kb) transcripts (Fig. 1) was often observed with Fny-CMV clone. Lowering the incubation temperature to 15 C (12) did not overcome this problem (Fig. 1). Both Fny-CMV and S-CMV riboprobes hybridized with the control strains CMV-D and CMV-S of the respective subgroups used in this study in either low- (incubation at 42 C) or high-stringency (incubation at 63 C) conditions. As expected from sequence data, RNA 3 riboprobes recognized the RNAs 3 and 4 of strains within their subgroup but hybridized poorly with genomic RNAs 1 and 2 (Fig. 2). At low stringency, a higher level of cross-hybridization with CMV strains belonging to the other subgroup was observed for the CMV-S riboprobe (not shown). However, at high stringency, CMV strains belonging to the different subgroups became clearly distinguishable (Fig. 3). Therefore, hybridization at 63 C was adopted as the routine procedure during the analysis of cultivated crops. TNA extracts from weed samples were always hybridized at both stringency conditions. D-CARNA 5 riboprobe was used only at low stringency. None of the three probes hybridized with TNA extracts from healthy control plants (tomato, celery, and zucchini squash), nor was the extent of hybridization changed when the sample contained 8 M urea and bromophenol blue.

Detection of CMV and CARNA 5 in cultivated crops. In Emilia-Romagna, 51 tomato samples were collected from six different fields. Figure 4 shows the distribution of isolates hybridizing with the two CMV riboprobes. Sequences specific to CARNA 5 were recorded in seven of nine tomato samples collected from field no. 5 and in all samples from a field near Anzola Emilia. Although plants from both fields apparently showed typical lethal necrosis symptoms (6), the helper strain of CMV seemed to be different, because positive signals were obtained with CMV-S riboprobe in field no. 5 and with CMV-Fny in the other. Plants from fields 1 to 4 showed fernleaf symptoms, and none of them had CARNA 5 sequences.

In Campania, 552 tomato samples were collected, 292 of which were collected in 1989, mainly in the provinces of Salerno and Caserta. Samples were selectively collected from plants showing severe fernleaf and/or fruit necrosis. All plants proved to be infected by CMV, which in about 95% of the cases strongly hybridized with the CMV-Fny riboprobe. CARNA 5 sequences were detected only in plants affected by fruit necrosis, but never in association with fernleaf-shoestring. This CARNA 5, provisionally named Tfn-CARNA 5, had an apparent size of about 0.38 kb under fully denaturing conditions (21) (Fig. 5) and proved to be nonnecrogenic in tomato. Instead, it attenuated symptoms induced either by the natural CMV helper isolate (CMV-Tfn) or by CMV-1 (A. Crescenzi and D. Gallitelli, *unpublished*). About 5% of the samples showed no clearly defined symptoms, such as necrotic rings on the fruit surface, marginal necrosis and/or upward rolling of leaflets, or stunting of the whole plant or of the shoot tip only. In these samples, viral nucleic acid extracts gave hybridization signals with both CMV riboprobes with no clear-cut symptom-strain relationship. The symptomatology was often complicated by the presence of mixed infections with alfalfa mosaic virus, potato virus Y potyvirus, and tomato mosaic tobamovirus.

The CMV-Fny riboprobe selectively detected CMV in 10 samples of celery with severe mosaic and in 20 of zucchini squash with mosaic and leaf and fruit malformations, whereas CMV-S hybridized strongly with TNA extracts from pepper (25 samples) and eggplant (three samples).

In the southeastern regions (Basilicata and Apulia), tomato samples were collected mainly from the Jonian coastal area, where lethal necrosis had occurred both in 1988 and 1989. In 1990, the disease was sporadic, because the number of tomato fields planted in the area had been severely reduced as a consequence of the epidemics of the preceding years. Other sampled areas of Apulia were the provinces of Brindisi, Foggia, and Lecce, where tomato fruit necrosis had been recorded sporadically in 1988–1989 and in 1990. About 350 tomato samples were collected, 80% of them in 1989. In Basilicata, 1990 sampling was done preferentially at Metaponto around the Cobas field, the site of the 1989 SPT. In both 1989 and 1990, lethal necrosis was constantly associated with the presence of CARNA 5 sequences and with a helper CMV isolate belonging to the S subgroup. The CMV-PG involved in the 1988 epidemics from the same Metaponto area (4,8) also belonged to the S subgroup.

Celery is a common winter crop (September through May) in Metaponto and is usually infected by CMV and celery mosaic potyvirus. A diseased celery plot planted near the Cobas field was surveyed for the presence of CMV in 1990 by collecting 25 samples monthly from January to June in different areas of the plot. In all samplings, the CMV isolate detected belonged to the WT subgroup. CARNA 5 sequences were consistently found associated with this CMV isolate, provisionally named CMV-CR. When inoculated into Rutgers tomato seedlings, CMV-CR containing CR-CARNA 5 induced severe stunting and upward rolling of the leaflets but not lethal necrosis. CR-CARNA 5 was the same size as PG-CARNA 5 (8).

CMV isolates that strongly hybridized with the CMV-Fny riboprobe were also

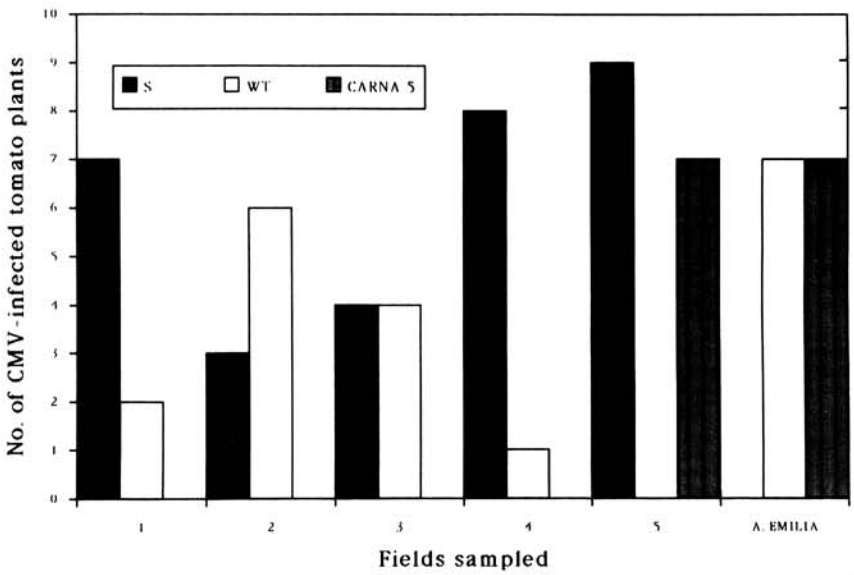

Fig. 4. Distribution of cucumber mosaic virus (CMV) strains WT and S in tomato samples collected from six different places in Emilia Romagna. CARNA 5 sequences were detected only in field no. 5 and in a field near Anzola Emilia.

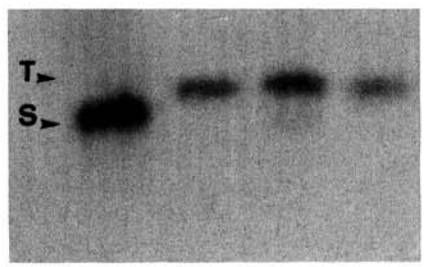

Fig. 5. Detection of CARNA-5 specific sequences in three different preparations of CMV-Tfn (T) and in one of CMV-S (S) used as reference. Migration from top to bottom.

found in melon, watermelon, and zucchini squash collected from the same area. Viral nucleic acid extracted from CMV particles purified from spinach collected in the Lecce province gave positive signals with the CMV-S riboprobe.

Detection of CMV and CARNA 5 in weeds. The occurrence and distribution of CMV in weeds were surveyed in Campania and Basilicata in 1989 and 1990.

In Campania, about 600 samples from different weeds were collected in winter, spring, and summer, and TNA extracts were assayed for the presence of viral and satellite sequences. The results shown in Table 1 and Figure 6 indicate that 45 samples from 20 different species hosted CMV, more frequently of the S subgroup (27 out of 45 samples). CARNA 5 sequences were detected in 11 instances, seven of which were S subgroup isolates. The method of White and Kaper (25) detected three instances where there were accumulations of dsCARNA 5 without the apparent presence of helper virus sequences. In these, the helper virus was not detectable either by molecular probe or by mechanical and aphid transmission to herbaceous hosts.

In Basilicata, 422 samples were collected in 1990 from weeds growing in five different localities: Cobas, the adjacent Pizzica, the more distant Serra Marina (about 5 km), Picoco Alto (12 km), and Venezia (15 km). As shown in Table 2, 42 samples from 21 different species were infected by CMV, which in two cases supported a CARNA 5. Ten of the weeds were not in the lists of CMV hosts and may be new records of natural hosts of the virus. Figure 7 shows the distribution of the strains belonging to the two CMV subgroups in relation to the place and time of sampling. CMV strains of the S subgroup were represented more frequently in weeds collected during winter but without a substantial difference between Cobas and the other fields. In spring, a progressive shifting of virus populations from S-type to WT-type strains was seen in the appearance of mixed infections. In summer, CMV had virtually disappeared except for a sample recorded at Picoco Alto.

DISCUSSION

The recurrence of CMV epidemics in Italian tomato crops over a period of three consecutive years enabled the collection of a large number of infected samples from different cultivated and wild hosts. These samples are used here for mapping the distribution of CMV strains in different regions. The molecular probes used for hybridization tests under high stringency allowed a clear-cut distinction between CMV isolates belonging to subgroups S and WT (17).

All samples tested were infected by single-virus isolates belonging to one of these subgroups, with the exception of a very few tomato and weed samples from Basilicata (Fig. 7). Whether these samples contained pseudorecombinants or mixed infections by two individual CMV strains has not been ascertained, but according to previous work in France (18,19) the latter possibility is more likely. Recently, mixed infections by individual CMV strains were also recorded in pepper in New York State and Bermuda (11).

Regardless of the geographical location, WT-type isolates in tomato were consistently associated with fruit necrosis but never with lethal necrosis, except for the single record at Anzola Emilia (Fig. 8). The reverse was true for S-type isolates, which prevailed in plants with lethal necrosis but were negligible in

Table 1. Weed hosts of cucumber mosaic virus (CMV) and CARNA 5 in Campania

Season Weed species	CMV subgroup	CARNA 5	No. of same recordings
Winter			
Antirrhinum majus	S	−	1
Eupatorium cannabinum[a]	S	+	1
Primula palinuri	S	−	1
Spring			
Borrago officinalis	WT	−	1
B. officinalis	S	−	2
Calendula officinalis	WT	−	2
Convolvulus spp.	WT	−	4
Daucus carota	...[b]	+	1
Lamium sp.	S	−	2
Mentha palustris	...	+	3
Raphanus raphanistrum	WT	−	3
Smyrnium sp.	S	−	1
Sonchus campfelii	S	−	3
Stellaria media	WT	−	3
Vicia sp.	S	−	3
Summer			
Cirsium arvense	...	+	1
Erodium malachoides	S	−	1
Mentha piperita	S	+	1
Mercurialis annua	S	+	2
M. annua	S	−	3
M. annua	WT	+	1
Ranunculus spp.	WT	−	2
Smilax aspera	S	+	1
Smyrnium perfoliatum	S	+	1
Solanum nigrum	WT	−	2
S. nigrum	S	−	3
Sonchus oleraceus	S	+	1

[a]Possible new CMV host species.
[b]No CMV infection detected.

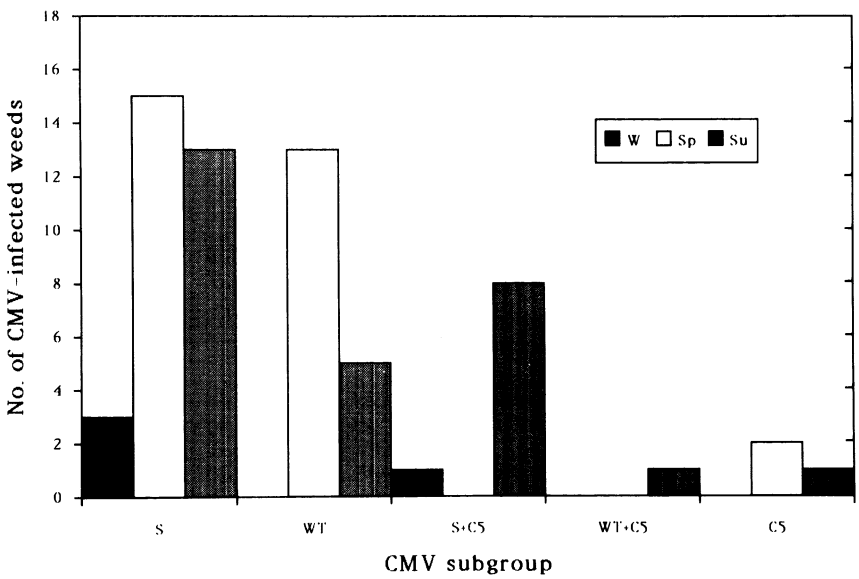

Fig. 6. Distribution of cucumber mosaic virus (CMV) strains of the WT and S subgroups in weeds collected in Campania. CARNA 5 (C5) was detected in association with strains of either group (S+C5, WT+C5) and also when viral sequences were no longer detectable. W = Winter, Sp = Spring, Su = Summer.

Table 2. Weed hosts of cucumber mosaic virus (CMV) and CARNA 5 in Basilicata

Season Weed species	CMV subgroup	CARNA 5	Locality
Winter			
Borago officinalis	S	−	Cobas
Calamintha nepeta[a]	WT	−	Pizzica
Carduus sp.[a]	WT	−	Pizzica
Carduus sp.[a]	S	−	Venezia
Chrysanthemum coronarium	S	−	Serra Marina
C. coronarium	S	−	Venezia
Cichorium intybus	S	−	Pizzica
Cirsium sp.	S	−	Venezia
Composite, undetermined	WT	−	Venezia
Composite, undetermined	WT	−	Serra Marina
Composite, undetermined	S	+	Cobas
Composite, undetermined	S	−	Picoco Alto
Foeniculum piperitum[a]	S	−	Cobas
Picris hieracioides	S	−	Serra Marina
Reichardia picroides[a]	S	−	Pizzica
Satureja juliana[a]	S	−	Picoco Alto
Spring			
Calamintha nepeta[a]	MIX[b]	−	Pizzica
C. nepeta[a]	WT	−	Picoco Alto
Calendula officinalis	S	−	Serra Marina
Carduus pycnocephalus[a]	WT	−	Venezia
Centaurea duriaei[a]	S	−	Picoco Alto
Composite, undetermined	WT	−	Cobas
Composite, undetermined	WT	−	Cobas
Composite, undetermined	MIX	−	Venezia
Composite, undetermined	WT	−	Picoco Alto
Convolvulus arvensis	S	−	Cobas
Diplotaxis tenuifolia	S	−	Serra Marina
Echium vulgare	WT	−	Serra Marina
Erigeron canadensis	S	−	Venezia
Inula graveolens[a]	WT	−	Cobas
Pallenis spinosa[a]	WT	−	Serra Marina
P. spinosa[a]	WT	−	Venezia
Picris hieracioides[c]	MIX	−	Cobas
P. hieracioides	WT	−	Cobas
P. hieracioides	WT	−	Pizzica
P. hieracioides	WT	−	Serra Marina
Reichardia picroides[a]	MIX	−	Venezia
R. picroides[a]	S	+	Cobas
R. picroides[a]	S	−	Picoco Alto
R. picroides[a]	WT	−	Picoco Alto
Summer			
Calamintha nepeta	WT	−	Picoco Alto

[a]Possible new CMV host species.
[b]The sample hybridized with both riboprobes.
[c]Recorded twice.

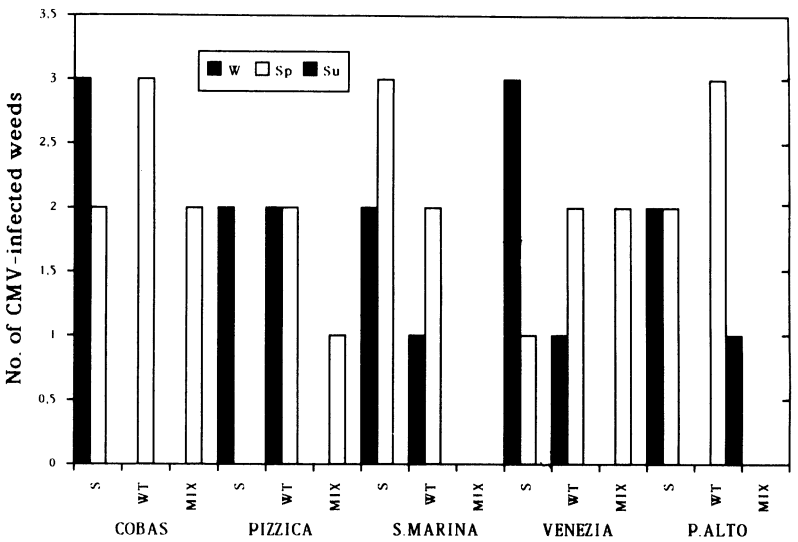

Fig. 7. Distribution of cucumber mosaic virus (CMV) strains belonging to WT and S subgroups in weeds collected in Basilicata in 1990. Samples hybridizing with both riboprobes were placed in the MIX category. W = Winter, Sp = Spring, Su = Summer.

those affected by fruit necrosis (Fig. 8).

Considering the kinds of disease prevailing in Basilicata and Campania and the seasonal distribution of CMV types in weeds, the question arises of whether and to what extent weeds are major sources of inoculum for CMV outbreaks in tomato. The data from Basilicata seem to support the notion that weeds play a key role in determining CMV epidemics for the S-type isolates, which predominate in wild plants throughout winter and are associated with lethal necrosis. Their absence in summer could be explained by the natural disappearance of some weeds in this season and by the self-destructive nature of the lethal necrosis in tomato. As infected tomato plants die, there is a strong reduction of inoculum available in the environment, and only some weeds are able to maintain the virus (probably in their seeds) until next autumn. WT-type strains appear in the spring when natural inoculation of tomato crops has already taken place. No wonder then, that WT-type strains are common in late spring and summer crops (melon, watermelon, zucchini squash) and in celery, which carries them through the autumn.

The situation in Campania is different and more difficult to reconcile with the above pattern. Although S-type strains occur in weeds at least as much as WT-type strains (Fig. 6), the latter predominate widely and are about the only ones to be found in tomato crops. This does not indicate that weeds do not represent inoculum sources; rather, it suggests that in Campania other crops may act as preferential virus reservoirs and that perhaps WT strains are transmitted more efficiently by aphids.

CARNA 5 sequences are either quite rare in weeds or below the level of detectability. Including previous records (1,5), the number of weeds recognized as natural hosts of CARNA 5 in Italy is now 15. This confirms that cultivated species are the largest natural reservoirs of CARNA 5 sequences in the areas stricken by CMV and probably play a major role in its epidemiology.

As to the possible impact of the SPT on the environment, Figure 7 shows that the number of weeds infected with S-group CMV in the Cobas field was comparable with that of other places, including those of locations far from it (i.e., Venezia and Picoco Alto). Moreover, this type of virus did not infect other crops (celery, melon, watermelon) growing in the immediate vicinity of the Cobas field. Therefore it seems that, at least in 1989–1990, CMV-S or its satellite RNA did not spread from protected tomato plants to other vegetable crops to a detectable level, even though the protected tomato crop was heavily infested by aphids (5). Tien and Wu (24) have reported that mild CMV strains used as biological control agents (BCAs)

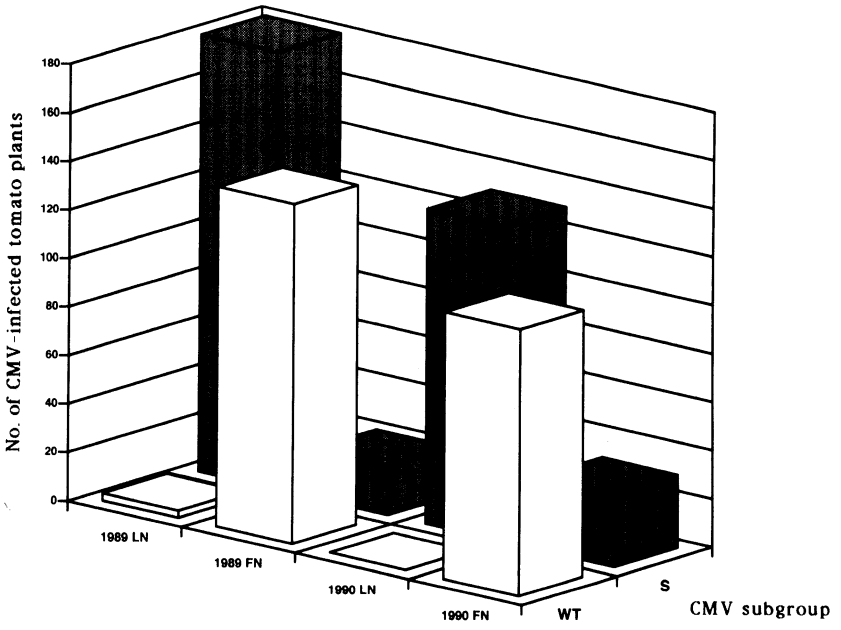

Fig. 8. Association of cucumber mosaic virus (CMV) subgroup strains WT and S with lethal necrosis (LN) and fruit necrosis (FN) syndromes in tomato samples after 2-yr collections in four Italian regions.

are generally transmitted with lower efficiency by aphids.

If BCAs will be used in the future, monitoring after release may be done in several ways, i.e., testing on suitable indicator plants, RNAse protection assay (16), temperature gel gradient electrophoresis (22,23), and whenever possible, nucleic acid sequence determination. The indications so far obtained on the dissemination of BCAs in the environment are encouraging. Moreover, the indirect protection of untreated plants exerted by plants inoculated previously (5) could help reduce the amount of BCAs released in the environment.

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