Identification of Turnip Mosaic and Cauliflower Mosaic Viruses Naturally Infecting Collards

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ABSTRACT

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Two viruses, cauliflower mosaic (CaMV) and turnip mosaic (TuMV), isolated from naturally infected collards, were identified by host range, physical properties, inclusion bodies, and serology. Prominent vein chlorosis and banding were associated with CaMV infections. TuMV was isolated from collards with very mild mottle and small chlorotic ring spots to severe mosaic and blisters. The collard isolates of TuMV differed from the type isolate in that they infected legumes (*Lupinus albus, L. angustifolius*, *Pisum sativum*, and *Phaseolus vulgaris* 'Bountiful'). The accuracy of two serodiagnostic tests for detecting TuMV was compared with that of bioassays. Latex agglutination serologic tests were more sensitive than sodium lauryl sulfate agar diffusion serologic tests and were useful in detecting TuMV from field plant samples of collards, turnips, and mustard. Inspection for virus-induced inclusions was a useful tool for diagnosing CaMV infection in collards but was less effective for TuMV.

Additional key words: *Brassica oleracea* var. *acephala*, legume strain of turnip mosaic virus

Collards (*Brassica oleracea* var. *acephala* DC.), a crucifer with high nutritive value usually used as a green leafy vegetable, are a commercially important cash crop in addition to a home garden vegetable in the southeastern United States. During early spring of 1979, viruslike symptoms such as severe mosaic with dark green blisters and/or prominent veinbandings were observed on a few plants in Colquit County in south Georgia. Within 3 wk of the first observation, disease had spread to 60% of all field plants examined. Outbreaks of mosaic disease in collards were also observed in South Carolina (O. W. Barnett, *personal communication*). Preliminary observations suggested that two viruses—turnip mosaic (TuMV) and cauliflower mosaic (CaMV)—were associated with the infected collards (5).

The literature on viruses that infect collards is limited and somewhat obscure. Collard and kale share the same botanical name and were both classified earlier as *B. oleracea* var. *viridis*. LeBeau and Walker (8) and Provvidenti (9) demonstrated that collard cultivars are experimental hosts for TuMV. While this manuscript was being prepared, a report (2) identified TuMV naturally infecting collards in Brazil. However, no report on CaMV infecting collards is available.

Because collards are grown throughout the year in Georgia, primarily in the turnip and mustard belt, virus infection in collards may threaten the commercial mustard and turnip crops. Our aim was to identify and characterize the viruses in collards and to develop rapid, sensitive procedures for detecting these viruses.

MATERIALS AND METHODS

Source of virus isolates and plant samples. The TuMV isolate 4C we used was isolated from a collard plant in Colquit County, GA. To assure the purity of this isolate, we passed it through *Lupinus albus* L. (white lupine), followed by three successive transfers via local lesions on Burley 21 tobacco. A previously identified isolate, TuMV-P (isolated from pea), was kindly supplied by R. Provvidenti (Cornell University). Because TuMV isolates do not infect *Datura stramonium* L. and have lower thermal inactivation points than CaMV, we obtained a pure isolate of CaMV by heating (70 C for 10 min) sap from infected collard plants, inoculating *D. stramonium*, and selecting local lesions. Both isolates were maintained in Tendergreen mustard and collards.

Eight commercial fields and 11 home gardens in eight counties in Georgia were examined for prevalence of viruses in collards. Leaves from individual plants were placed in a polyethylene bag in an ice chest, coded, and stored in a cold room (4 C) until indexed. These samples were divided into four lots, consisting of samples showing severe mosaic, prominent veinbandings, very mild mottle and/or a few chlorotic ring spots, or a normal appearance.

Determining virus infections. Samples were tested for virus infection by three methods: indexing on diagnostic hosts, cytology of induced inclusions, and serodiagnosis.

Indexing. For mechanical inoculations, we triturated a portion of the leaves with 0.02 M potassium phosphate buffer, pH 7.2, containing 1% Celite (1 g of tissue per milliliter). We rubbed the resulting sap onto Carborundum (600 mesh)-dusted leaves of *B. perviridis* L. H. Bailey 'Tendergreen', *Chenopodium amaranticolor* Coste & Reyn., *Gomphrena globosa* L., *Cucurbita pepo* L. 'Early Yellow Summer Crookneck', *Nicotiana tabacum* L. 'Burley 21', and *D. stramonium*, hosts used for preliminary diagnosis.

Plants used in the host range tests were grown from seeds in a soil-vermiculite (3:1) mixture and maintained in a greenhouse at about 20–24 C. Sap from the plants in the host range tests was indexed on Tendergreen mustard 3 wk after inoculation.

Inclusions. We examined a second portion from each sample for the presence of virus-induced inclusions. Epidermal strips from collards, mustard, and turnips were stained with orange-green (calcomine orange-Luxol green) and/or azure A combinations as described by Christie and Edwardson (1) and observed under a Zeiss Ultraphot II microscope. For permanent preparation, stained strips were differentiated in ethanol, passed through a graded series of ethanol-xylene, and mounted in Canada balsam.

Serology. Antiserum (TuMV-F 744) against TuMV was kindly supplied by D. E. Purcifull (University of Florida). Agar double diffusion tests with sodium lauryl sulfate (SDS) denatured antigen were performed in agar gels prepared in

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deionized water containing 0.8% Noble agar, 1% sodium azide, and 0.5% SDS. We prepared antigens by extracting 2 g of leaves in 2 ml of deionized water to which 2 ml of 3% SDS was added after triturating. Plates were arranged as described previously (10) and were observed for precipitin reaction after 20 and 48 hr of incubation at room temperature.

The latex agglutination test (LAT) was performed by a capillary procedure as described earlier (7), except that the crude sap extract (antigen) was prepared by triturating about 0.5 g of leaf tissue per milliliter of buffer (0.1 M tris-HCl-saline, pH 7.2). Occasionally, the extract was centrifuged at 8,000 *g* for 10 min to clear debris.

Serologic tests from CaMV were performed in Ouchterlony double-diffusion plates prepared in deionized water containing 0.8% ion agar 2, 0.8% NaCl, and 1% sodium azide. Virus antigen from crude sap was concentrated by a procedure described earlier (6). Plates were handled similarly to those of TuMV.

RESULTS

Symptomatology. Symptoms in field-grown collards, inclusion bodies in epidermal strips, and symptoms induced on indicator hosts suggested that more than one virus was involved in the collard mosaic problem. Symptoms observed in naturally infected collards varied in severity but were usually one of two types (Fig. 1) or combinations of these types. Leaf vein symptoms such as mild vein chlorosis, veinbanding, and a leathery appearance were called type 1. Symptoms of mild light green or chlorotic mottle, chlorotic spots and ring spots, puckering, and dark green blisters were called type 2.

In the spring, type 1 was predominant; however, in summer with higher temperatures, type 2 was predominant. Type 1 isolates induced systemic veinbanding, severe leaf deformations, and stunting in Tendergreen mustard and purple top turnip and a few local lesions on *D. stramonium*. Typical type 2 isolates induced severe mosaic in Tendergreen mustard and purple top turnip, chlorotic stippling on squash, and local lesions in *Chenopodium amaranticolor*, Burley 21 tobacco, and *G. globosa*. Type 1 isolates were separated from mixed infections by heating the sap (70 C for 10 min) before inoculating crucifers or *D. stramonium*. Pure cultures of type 2 isolates were recovered by passage through *L. albus* followed by successive local lesion transfers through Burley 21 tobacco.

In greenhouse tests, experimentally inoculated collard cultivars Georgia Green and Vates showed mild vein chlorosis and bandings with type 1 isolates and mottle, chlorotic stippling, and distortion with type 2 isolates. Mixed infections with both isolates did not enhance symptoms much. Symptoms induced by either isolate were always less severe than those observed in the field.

Serology. Initial serologic tests using Ouchterlony double-diffusion and SDS agar immunodiffusion revealed that type 1 isolates reacted against CaMV antiserum and type 2 isolates reacted with TuMV antiserum. SDS agar immuno-diffusion tests were useful in detecting TuMV from infected collards, mustard, and turnips directly from field samples. Samples from field- or greenhouse-grown crucifers infected with CaMV reacted only occasionally in double-diffusion tests. However, the presence of CaMV was always confirmed when the antigens were concentrated from such samples (6). Samples collected in early spring gave a higher percentage of positive tests that did not need antigen concentration.

Inclusion bodies. Cytochemical tests were extremely useful in determining type 1 infections in crucifers. All 35 samples that reacted with CaMV antiserum contained typical vacuolated, ovoid, cytoplasmic inclusions characteristic of caulimovirus group infections (Fig. 2A and B). In infected collard epidermal strips, these inclusions were smaller and less vacuolated (thus more crystalline-looking) than the large, ovoid, vacuolated inclusions observed in turnip and mustard epidermal strips infected with the same isolate. Numerous small crystalline intranuclear inclusions that stained green with azure A were observed in infected tissue; however, a few were occasionally observed in healthy tissue.

Diagnosis of type 2 infection in field samples of collards by inclusions was difficult. Of 50 attempts, only eight samples showed inclusions, although all had type 2 infection as determined by other tests. Collards, turnips, and mustard plants infected with type 2 isolates in the greenhouse showed cylindric inclusions in the cytoplasm (Fig. 2C) and were typical of potyvirus group (TuMV) infections.

Characterization of collard isolate of TuMV. Host range studies indicated that TuMV-4C (type 2 isolate) and other similar isolates from collards, mustard, and turnip differed from the reported type strain in host range in that several legume species were susceptible. Isolate

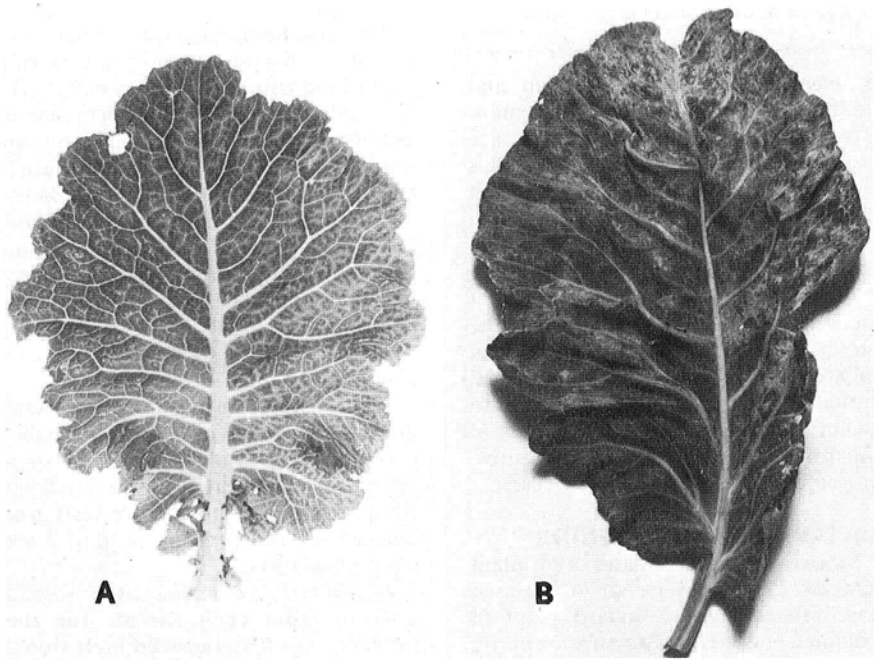

Fig. 1. Symptoms of cauliflower mosaic virus **(A)** and turnip mosaic virus **(B)** in collards.

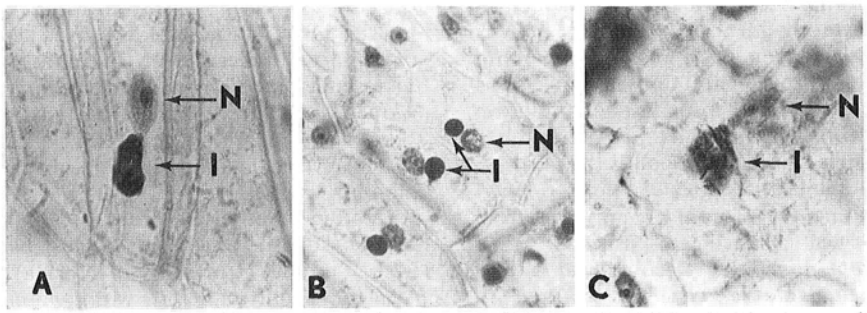

Fig. 2. Inclusions of cauliflower mosaic virus in turnip **(A)** and collard **(B)** and of turnip mosaic virus in collard **(C)**; N = nucleus, I = inclusions (A and B, ×1,000; C, ×800).

TuMV-4C was compared with a legume isolate (TuMV-P). Isolate TuMV-4C induced local and systemic chlorotic spots and ring spots, followed by top necrosis and death, in *L. albus*; mild mosaic and chlorotic spots, with severe reduction in plant size, in *L. angustifolius*; severe distortion in *Cicer arietinum* L.; chlorotic local lesions (in one of three trials) in *P. vulgaris* 'Bountiful'; small necrotic lesions in *P. vulgaris* 'Topcrop'; no symptoms in *Pisum sativum* 'Alaska' and 'Little Marvel' or *Vicia faba* L.; and local chlorotic lesions in *Cassia occidentalis* L. and *Cassia obtusifolia* L. Isolate TuMV-P differed by causing severe mosaic in the pea cultivars and by not infecting Bountiful, Topcrop, or faba beans.

Cucurbita pepo 'Early Yellow Summer Crookneck' showed chlorotic stippling after being inoculated with TuMV-4C, but virus could not be detected by either back-inoculation to turnip or by serologic tests. No symptoms were produced on nor could virus be recovered from *Raphanus sativus* L. 'Redglobe', *Cajanus cajan* (L.) Huth, *Cyamopsis tetragonoloba* (L.) Taub., *Desmodium canum* (Gmel.) Schinz & Thel., *Glycine max* (L.) Merr. 'Bragg', *P. calcaratus* Roxb., *Trifolium pratense* L. 'Kenland' and 'Clone L-12', *T. repens* L. 'Ladino', *N. glutinosa* L., *Cucumis sativus* L. 'Straight-8', and *Citrullus vulgaris* Schrad.

Because TuMV isolates from collards infected legumes systemically, virus identity and relationship with common legume viruses were determined by latex agglutination serology. Latex conjugated with antiserum against bean yellow mosaic virus (BYMV), clover yellow mosaic virus (CYMV), clover yellow vein virus (CYVV), peanut mottle virus (PMV), and white clover mosaic virus (WCMV) failed to agglutinate with TuMV antigen (sap prepared from infected legumes and crucifers). Sap from plants infected with BYMV, CYMV, CYVV, PMV, or WCMV also did not agglutinate TuMV antibody-coated latex conjugates. However, positive agglutination reactions were always observed in all the homologous combinations used.

Efficiency of serologic tests in detecting TuMV. The efficiency with which LAT, SDS immunodiffusion, and bioassay detected TuMV directly from field collards, mustard, and turnips is given in Table 1. The LAT test failed to detect one of 90 and the SDS immunodiffusion test six of 90 samples that reacted positively in bioassays on turnip and mustard. None of the samples that tested negatively by bioassay gave a positive result with either serologic test. The usefulness of LAT for detecting TuMV in composite samples was determined by combining one infected leaf with one, four, nine, 19, and 39 healthy leaves. Detection from simulated samples was consistently successful with one infected leaf in five leaves, and occasional positive assays were obtained with one infected leaf in 10 leaves.

Virus distribution. The distribution of TuMV and CaMV in Georgia is indicated in Table 2. Most infected collards in southern and central Georgia are infected with TuMV. The reverse situation occurs in northern Georgia, where CaMV causes the predominant virus disease.

DISCUSSION

In 1973, Demski (3) reported TuMV and CaMV infecting turnips and mustard plants in Georgia but did not observe viruslike symptoms on collards. Other observations of collards (O. W. Barnett, *personal communication*) before 1978 did not indicate that virus diseases were a major problem in the production of this crop. In the last few years, a high percentage of collard plants growing in commercial fields and home gardens have been observed with symptoms typical of virus diseases. We have identified two viruses (TuMV and CaMV) by host range, cytology, and serology as the cause of the collard mosaic problem.

Based on host range studies, the TuMV strain most frequently isolated from collards is distinct from the type strain because it infects a number of different legume species. Isolation and characterization of similar strains of TuMV have been reported from *Sinapis alba* (*B. alba*) in Morocco (4) and from peas in New York (9). Only minor differences in host range of these isolates were noted. Based on the reaction of differential hosts (particularly Chinese cabbage cultivar Michihli), our isolate (TuMV-4C) was characterized as similar to TuMV-C (R. Provvidenti, *personal communication*).

TuMV was recently reported to infect collard in Brazil (2). Although this isolate was not available for direct comparison with our isolates, the host range and symptom expression in the same hosts appear to differ (the Brazilian isolate is symptomless in collards).

Viruses are difficult to identify based on symptoms only. Frequently, TuMV and CaMV were isolated from plants with characteristic veinbanding symptoms often attributed to CaMV infections. Similarly, CaMV was also isolated from severely mottled plants. Double-diffusion serology using CaMV antiserum and sap extracted directly from field samples was not reliable unless sap was pretreated (clarified and concentrated with polyethylene glycol-NaCl), which may indicate that only small amounts of virus particles were diffusible from untreated sap. Recently, Shalla et al reported (11) that virions of some CaMV isolates are packed in an inclusion matrix and few particles may be dispersed in cytoplasm. In such cases, treatments that disrupt the inclusion matrix would provide more diffusible antigen (virus particles) in sap for serologic reactions.

We observed that CaMV-induced inclusions in epidermal strips from collards were smaller and more granular than those from infected turnips. This supports the recent report (11) that CaMV inclusions, unlike potyviruses, are determined by the interaction of both the virus and the host. Inclusions were a valuable aid in identifying double infections, because CaMV could not always be determined by serologic test directly from sap but almost always induced inclusions.

Bioassay from field-grown collards is the most sensitive method for detecting

Table 1. Accuracy of serodiagnostic tests for detecting turnip mosaic virus (TuMV)

Plant species	No. of samples	Positive test samples[a]		
		SDS agar	LAT	Bioassay
Collard				
With symptoms	60[b]	52	55	55
Symptomless	20	2	4	5
Mustard	15	15	15	15
Turnip	15	15	15	15
Healthy (check)	10	0	0	0

[a] SDS agar = double-diffusion test with agar containing SDS; LAT = latex agglutination test. Bioassay on turnip and tobacco.
[b] Five samples that were negative for TuMV tested positive for cauliflower mosaic virus.

Table 2. Distribution of turnip mosaic virus (TuMV) and cauliflower mosaic virus (CaMV) in collards in Georgia

Location	No. of samples indexed[a]	Virus-infected samples		
		TuMV	CaMV	TuMV + CaMV
Northern Georgia	40[b]	8 (20%)	35 (87%)	6 (15%)
Central Georgia	30	26 (87%)	13 (43%)	9 (30%)
Southern Georgia	130	120 (92%)	45 (35%)	35 (27%)

[a] Samples were collected from May to December 1979. Indexing procedure was a combination of bioassay, latex agglutination test, and inclusion light microscopy.
[b] Three samples tested negative for both viruses.

virus infection; however, LAT is a sensitive, efficient, inexpensive, simple, and rapid test for detecting TuMV. The use of LAT is especially appropriate when a large number of samples must be assayed.

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