AN UNUSUAL STRAIN OF TOBACCO MOSAIC VIRUS FROM *PLUMERIA ACUTIFOLIA**

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Virus-like symptoms were observed on leaves of frangipani (*Plumeria acutifolia* Poir.) from several gardens in South Australia and New South Wales. The leaves showed chlorotic ringspots or mosaic and were often distorted (Fig. 1). As far as we are aware no viruses from frangipani have been previously isolated. This communication is concerned with the isolation and characterization of an unusual strain of tobacco mosaic virus (TMV) from frangipani.

Leaves of frangipani showing disease symptoms were ground with a pestle and mortar in a 2·5% aqueous solution of nicotine (Eastman Organic Chemicals, Rochester, N.Y.) and mechanically inoculated to *Datura stramonium* L. Leaves of the inoculated plants developed chlorotic lesions, some with necrotic centres, after 2–3 weeks (Fig. 2). The virus (FV) was readily transmitted to *Nicotiana glutinosa* L. whose leaves developed water-soaked, blotchy lesions 2–3 weeks after inoculation (Fig. 3). Both Turkish Samsun and Blue Pryor tobacco (*Nicotiana tabacum* L.) appeared immune to FV as no symptoms were observed and the virus was not recovered by back-inoculation to *D. stramonium*. Local lesions were produced on inoculated leaves of *Nicotiana clevelandii* Gray, *Gomphrena globosa* L., and *Chenopodium quinoa* Willd. but not on *Chenopodium amaranticolor* Coste & Reyn.

As FV could not be identified from its host range, examination of virus particles in the electron microscope by the "quick dip" method (Hitchborn and Hills 1965) was carried out. Rigid, rod-shaped particles with a mean length of about 300 nm were seen in preparations from infected leaves of frangipani, *D. stramonium*, and *N. glutinosa*. These particles were indistinguishable from those seen in preparations of TMV. FV was purified from infected leaves of *N. glutinosa* by charcoal and DEAE-cellulose clarification, filtration through celite, and differential centrifugation (Francki and McLean 1968). The ultraviolet spectra of these preparations were characteristic of TMV, and rod-shaped particles with very little contaminating material were observed in the electron microscope (Fig. 4).

Antisera to FV were prepared in rabbits and were used to test the serological relationship of FV to some strains of TMV in precipitin tube tests. The results summarized in Table 1 indicate that FV is distantly related to the U1 strain of TMV and even more remotely related to the U2 strain. Both these strains of TMV have

* Manuscript received April 30, 1971.

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been described by Siegel and Wildman (1954). No serological reactions were detected between FV antigen and an antiserum prepared against a TMV strain from orchids (Francki 1966). Further serological studies were carried out by gel-diffusion tests

TABLE 1

SEROLOGICAL RELATIONSHIP BETWEEN FV AND THE U1 AND U2 STRAINS OF TMV

Results given are reciprocal of precipitation titres of sera recorded after incubation at 37°C for 1 hr followed by 4°C for 20 hr. In all the tests antigens were used at a concentration of 40 μg/ml. Values of O signify absence of visible precipitate at an antiserum dilution of one-half

Antigen	Anti-FV Serum	Anti-U1-TMV Serum	Anti-U2-TMV Serum	Non-immune Serum
FV	1024	256	64	0
U1-TMV	16	2048	256	0
U2-TMV	0	128	8192	0

using FV protein prepared by the acetic acid method (Fraenkel-Conrat 1957) and antisera to proteins of the TMV strains U1 and U2, tomato atypical mosaic virus (TAMV) strains Y and G, and Holmes' ribgrass (HR) strain of TMV (Knight 1963).

TABLE 2

AMINO ACID COMPOSITION OF FV PROTEIN

Results were calculated from five separate determinations on two independently prepared virus preparations. For each determination protein was hydrolysed for 24 and 72 hr and the amino acids separated and estimated as described by Zaitlin and McCaughey (1965). In addition to amino acid standards, hydrolysed protein of U1-TMV was run as an additional standard

Amino Acid	No. of Amino Acid Residues per Protein Subunit*		Amino Acid	No. of Amino Acid Residues per Protein Subunit*	
	FV	U1-TMV		FV	U1-TMV
Lysine	4	2	Glycine	9	6
Histidine	1	0	Alanine	14	14
Arginine	11	11	Cysteine	1	1
Aspartic acid and asparagine	17	18	Valine	13	14
			Methionine	0	0
Threonine	13	16	Isoleucine	11	9
Serine	14	16	Leucine	13	12
Glutamic acid and glutamine	16	16	Tyrosine	5	4
			Phenylalanine	7	8
Proline	4	8	Tryptophan	5	3

* Total number of residues per protein subunit equals 158.

Positive reactions were obtained between FV protein and antisera to Y-TAMV and U1-TMV but not with antisera to U2-TMV, G-TAMV, or HR. Van Regenmortel

(1967) has shown that U1-TMV is closely related serologically to Y-TAMV whereas U2-TMV is closely related to G-TAMV.

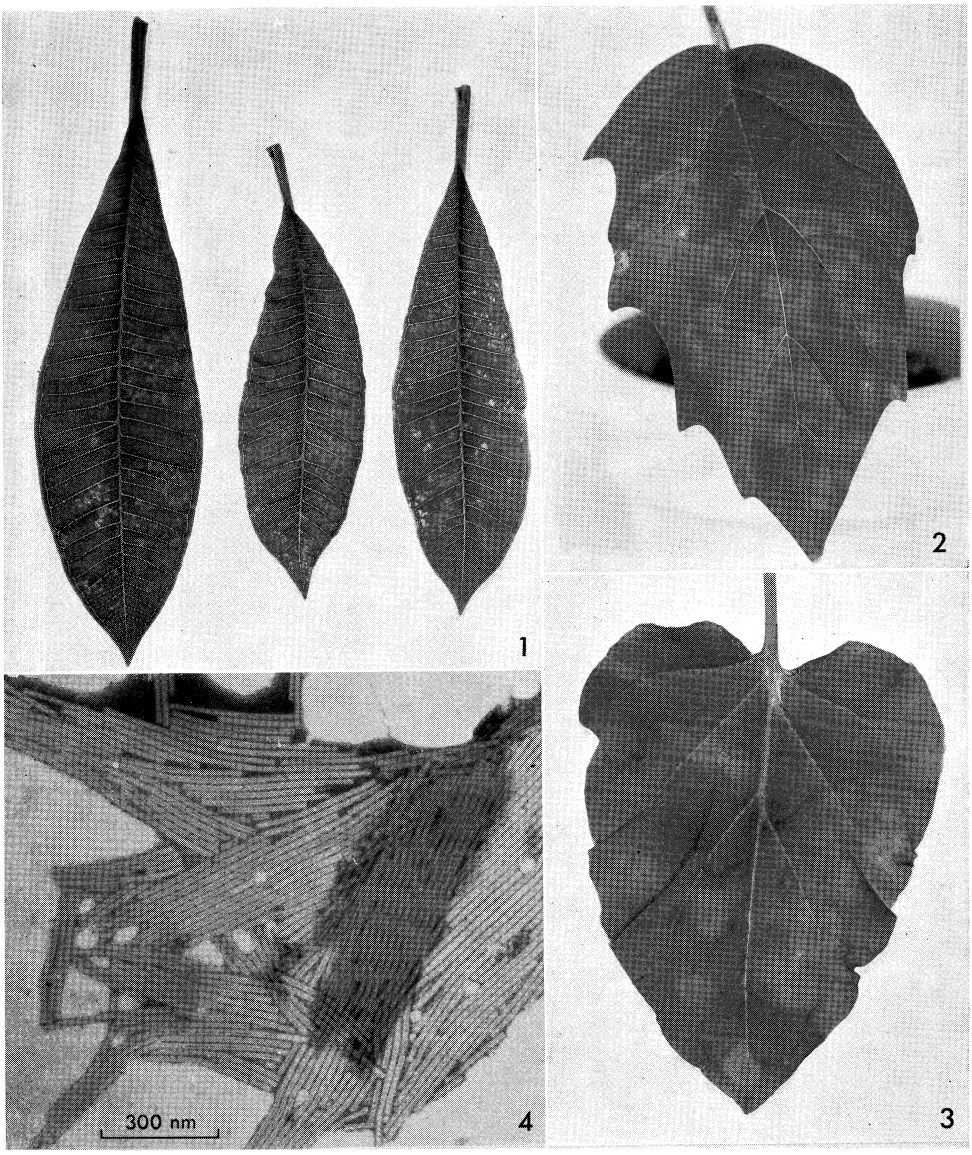

Fig. 1.—Symptoms on leaves of frangipani (*Plumeria acutifolia*) from which FV was isolated.

Fig. 2.—Symptoms on leaf of *Datura stramonium* mechanically inoculated with FV.

Fig. 3.—Symptoms on leaf of *Nicotiana glutinosa* mechanically inoculated with FV.

Fig. 4.—Electron micrograph of a purified preparation of FV stained with phosphotungstic acid, pH 6·8.

The amino acid composition of FV protein (Table 2) shows that it is TMV-like in that each subunit has 158 residues as do most strains of TMV (Knight 1963; Rees and

Short 1965; Chessin, Zaitlin, and Solberg 1967; van Regenmortel 1967; Kado, van Regenmortel, and Knight 1968). However, the exact amino acid composition of FV is significantly different from that of any described strain of TMV; its most striking features are low proline and high tryptophan contents (Table 2).

The particle morphology, serological properties, and amino acid composition of FV leave little doubt that it is a strain of TMV. However, it is easily distinguishable from other strains of TMV by its unusual host range and symptomatology and significant differences in its antigenic properties and the primary structure of its protein coat.

Acknowledgments

We thank Mr. S. R. Hockley for first drawing our attention to the disease symptoms on frangipani in South Australia; to Mr. H. Knuhtsen for a generous gift of antisera to the TMV proteins; and to Mrs. Fran Almli, Mrs. Ruth Smith, and Mr. A. Longo for excellent technical assistance. Some of the work reported was done by one of us (R.I.B.F.) while on leave, with financial support from the Australian–American Educational Foundation.

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