SHORT COMMUNICATION

FIG MOSAIC IN MEXICO AND SOUTH AFRICA

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SUMMARY

Different patterns of chlorotic to yellowish mottling and deformation were observed in leaves of field-grown fig trees in Mexico and South Africa. Potted rooted cuttings from both sources grown under glasshouse conditions displayed chlorotic blotching, vein clearing and banding of the leaves. Electron microscope observations of thin-sectioned tissues from symptomatic leaves showed that parenchyma cells of both Mexican and South African samples contained the double-membrane bodies (DMBs) typically associated with fig mosaic disease (FMD). Also, accumulations of filamentous semi-rigid and of very flexuous virus-like particles were observed in parenchyma or phloem cells, respectively. RT-PCR assays of total nucleic acids extracted from leaf tissues of both sources using primers designed to amplify the HSP70 gene of the putative closteroviruses Fig leaf mottle-associated virus 1 (FLMaV-1) and Fig leaf mottle-associated virus 2 (FLMaV-2) gave a positive response only for FLMaV-1. DMBs have been observed previously in the symptomatic Mexican fig source. However, our results are the first records of FMD and associated DMBs in South Africa and of both FLMaV-1 and an unidentified filamentous semi-rigid virus in Mexico and South Africa.

Key words: Ficus carica, fig mosaic disease, double-membrane bodies, fig leaf mottle-associated viruses, cytopathology, electron microscopy, RT-PCR.

Diseased fig trees (*Ficus carica* L.) were observed in 1988 at El Batan (State of Queretaro de Arteaga, Mexico) and, in 2006, in a farm at Calitzdorf (Little Karoo, Western Cape, South Africa). In each place, the trees showed different patterns of chlorotic to yellowish mottling and various types of leaf deformation, i.e a symptomatology closely resembling that described for fig mosaic disease (FMD), a putative virus-induced disorder (Condit and Horne, 1933).

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Earlier observations of thin-sectioned leaf tissues of the Mexican fig accession, had shown the presence in parenchyma cells of the so-called double-membrane bodies (DMBs) (Appiano *et al.,* 1995) consistently associated with FMD, originally observed by Bradfute *et al.* (1970) in figs and other plants affected by mite-transmitted diseases, and later described in detail (Martelli, 1991; Martelli *et al.* 1993). DMBs resemble particles of Pigeonpea sterility mosaic virus (PPSMV) and Maize red stripe virus (MRSV), both of which are representatives of a possible new genus of viruses with divided ambisense RNA genomes (Lava Kumar *et al.*, 2003; Skare *et al.*, 2006).

More recently, other viruses have been found in FMD-affected trees, two of which, Fig leaf mottle-associated virus 1 (FLMaV-1) and Fig leaf mottle-associated virus 2 (FLMaV-2), are members of the family *Closteroviridae* (Elbeaino *et al.,* 2006, 2007).

These findings prompted an investigation of diseased Mexican and South African fig accessions at the ultrastructural level and by RT-PCR for the possible presence of FLMaV-1 and FLMaV-2. For these studies, we used tissues from symptomatic leaves (Fig. 1A, Fig. 2A) of rooted cuttings from infected trees grown in a controlled environment glasshouse at 22-24°C.

Tissue fragments from veins and mesophyll of the discoloured areas of young leaves were processed for thin sectioning as described by Martelli and Russo (1984), i.e. fixation in 4% glutaraldehyde in 0.05 M phosphate buffer for 2 h at 4°C, post-fixation in 1% osmium tetroxide for 2 h, staining overnight in 2% aqueous uranyl acetate, dehydration in ethanol and embedding in low viscosity resin (Agar Scientifics, Stansted, UK). Thin sections were stained with lead citrate and viewed using a Philips Morgagni 282D electron microscope. Controls were leaf tissues from healthy fig seedlings processed as above.

The architecture of parenchyma cells of infected sources was fairly well preserved and did not differ much from that of healthy controls, except for an increased vacuolation of the cytoplasm. Nuclei were apparently normal whereas many chloroplasts were rounded and swollen, contained large starch grains, and had a reduced internal lamellar system. Mitochondria showed an extensive range of variations, resulting in more or

less severe derangement of the cristae, which were fewer than normal and dilated. The cytoplasmic membrane system was characterized by proliferation of the endoplasmic reticulum and of membranous vesicles of various sizes, that often accumulated next to the cell wall, giving rise to paramural inclusions. Such cytopathological features were present in both diseased fig accessions.

Parenchyma cells of the Mexican source contained DMBs with the same morphology (rounded to ovoid), size (90 to 160 nm in diameter) and overall appearance (Fig. 1B) as those previously observed in the same Mexican accession (Appiano *et al.* 1995; Appiano and Conti, 2001) and filamentous virus-like particles *ca.* 8 nm in diameter, that seemed to cluster preferentially next to chloroplasts (Fig. 1D). Comparable particles with a similar intracellular location have been observed previously in an Italian FMD-affected tree (Martelli *et al.*, 1993). No DMBs were seen in sieve tubes, although several did

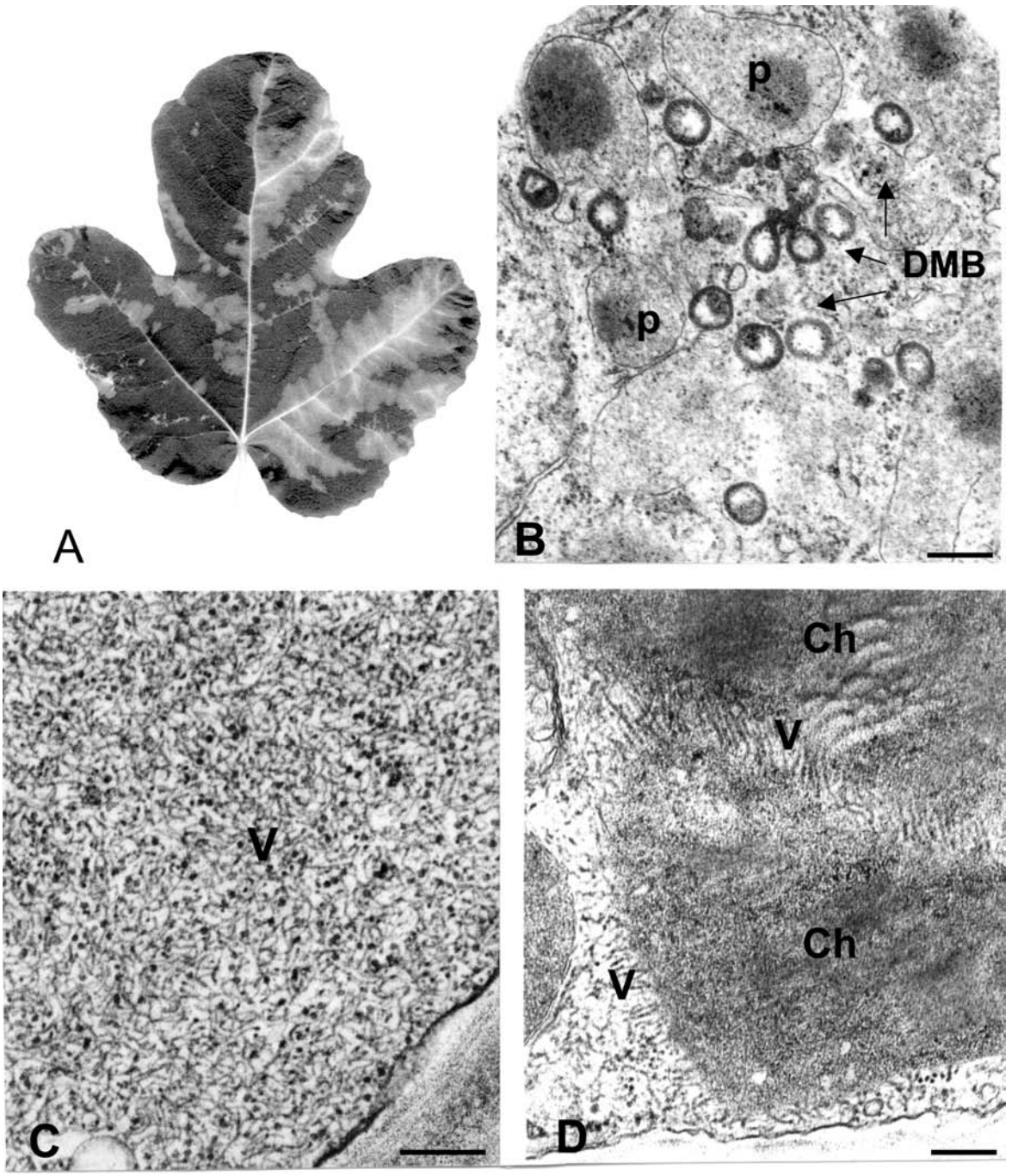

Fig. 1. A. Symptoms shown by a leaf of a greenhouse-grown rooted cutting from a FMD-affected Mexican fig accession. B. A group of double-membrane bodies (DMB) in a parenchyma cell. C. Very flexuous filamentous particles (V) filling the lumen of a sieve tube. D. Profiles of filamentous semi-rigid virus-like particles (V) surrounding chloroplasts (Ch) in a parenchyma cell. p, peroxisomes. Bars = 200 nm.

contain clusters of membranous vesicles and very flexuous, filamentous virus-like particles that often filled the cell lumen (Fig. 1C).

Similar flexuous particles but no DMBs were seen in phloem elements of the South African fig accession (Fig. 2D). In contrast, parenchyma cells contained bundles of filamentous particles like those present in the Mexican accession (Fig. 2E) and DMBs of two types, rounded to ovoid, 100 to 160 nm in size (Fig. 2B) and elongated, straight to slightly flexuous *ca.* 100 nm in diameter and up to or exceeding 1 µm in length (Fig. 2D). Comparable long DMBs have been observed previously in FMD-affected trees from Italy (Martelli *et al*., 1993).

RT-PCR assays for the identification of the filamentous particles present in the phloem of Mexican and South African samples were done using the virus-specific primers designed to detect the HSP70 nucleotide sequence of FLMaV-1 (Elbeaino *et al*., 2006) or FLMaV-2

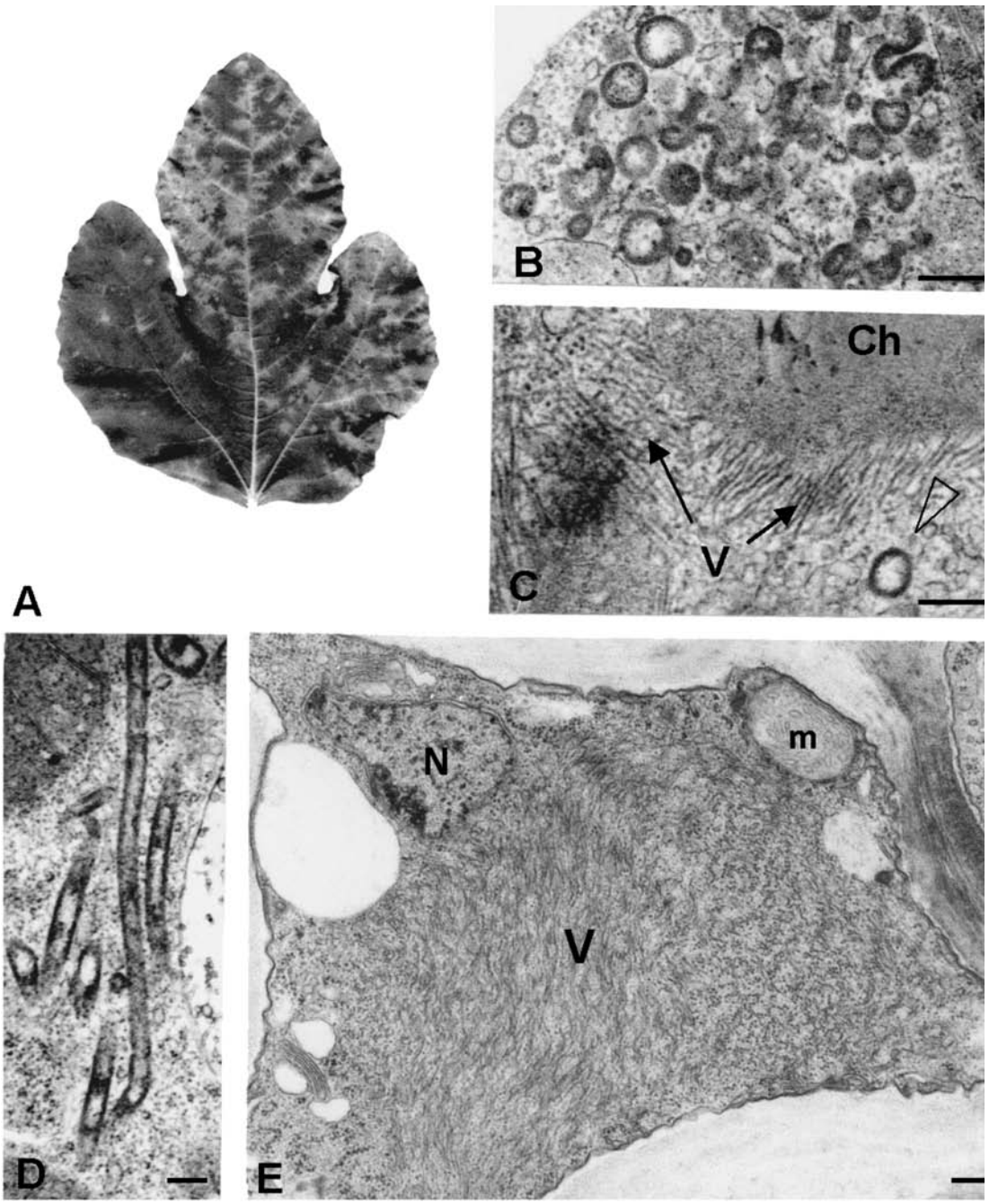

Fig. 2. A. Symptoms shown by a leaf of a greenhouse-grown rooted cutting from a FMD-affected South African fig accession. B. A group of globose double-membrane bodies (DMB) in a parenchyma cell. C. Profiles of filamentous semi-rigid virus-like particles (V) surrounding chloroplasts (Ch) in a parenchyma cell. Arrow head points to a DMB. D. Elongated slightly flexuous DMBs in a parenchyma cell. E. Very flexuous filamentous particles (V) in the cytoplasm of a companion cell. N, nucleus; m, mitochondrion. Bars = 200 nm.

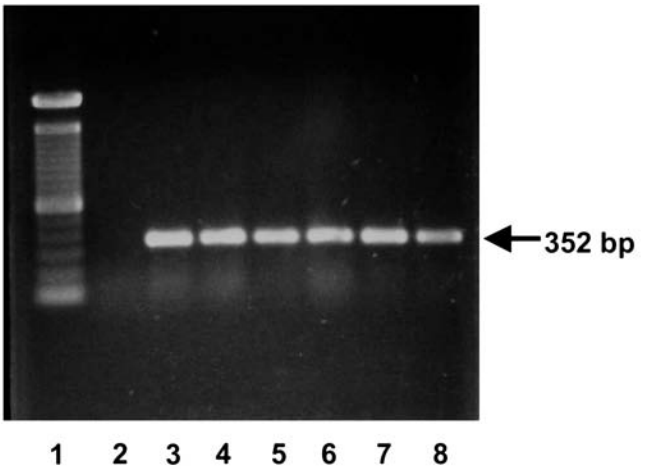

Fig. 3. RT-PCR amplification products obtained using FLMaV-1-specific primers on TNA extracts. Lane 1, DNA molecular weight marker; lane 2, healthy control; lane 3, Mexican FMD-affected fig accession; lanes 4-7 different South African FMD-affected fig accessions; lane 8, positive control.

(Elbeaino *et al.*, 2007) using as template total nucleic acids (TNAs) extracted from 100 mg leaf tissues ground in 1 ml grinding buffer (4.0 M guanidine thiocyanate, 0.2 M NaOAc pH 5.2, 25mM EDTA, 1.0 M KOAc and 2.5% w/v PVP-40). TNAs were recovered with a silica-capture protocol according to Foissac *et al.* (2001), resuspended in 120 µl RNase-free sterile water and cDNA was synthesized by random priming at 42°C for 1 h with MoMLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). PCR was done in a thermal cycler (Perkin-Elmer 7600), programmed for one cycle at 94°C for 4 min, followed by 35 cycles of 94°C for 30 sec, 58 °C for 30 sec, and 72°C for 40 sec. Final extension was at 72°C for 7 min.

TNA preparations from both Mexican and South African sources yielded amplified products of *ca.* 350 bp, the size expected for FLMaV-1 (Elbeaino *et al.* 2006) (Fig. 3). No amplicons were obtained when PCR was done with primers specific for FLMaV-2 (not shown).

Unlike for Mexico, there is no apparent record of FMD from South Africa. The present study has provided evidence that FMD-affected accessions from both countries are infected by at least three viruses: a still uncharacterized putative virus with enveloped particles (DMBs), an unidentified virus with filamentous semi-rigid particles, and the putative closterovirus FLMaV-1. In addition to Mexico and South Africa, this latter virus has been recorded from Italy (Elbeaino *et al.*, 2006), Tunisia (Nahdi *et al.*, 2006), California (Falk and Salem, 2006), Lebanon, Albania, Chile (T. Elbeaino, personal communication), and Greece (unpublished information). Its occurrence in widely separated geographical areas can be taken as an indication of an ubiquitous distribution.

Assuming that DMBs, due to their consistent presence in diseased figs, are involved in the aetiology of FMD (Martelli *et al.*, 1993), the frequent occurrence in symptomatic plants of other viruses (e.g. FLMaV-1 and the unidentified filamentous entity with a cytoplasmic localization) suggests that FMD is a complex disorder that may be caused by different infectious agents.

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Received April 16, 2007
Accepted June 8, 2007