A Fig Deal: A Global Look at Fig Mosaic Disease and its Putative Associates

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Description of Fig Mosaic Disease and its Associated Culprits

The common fig (*Ficus carica* L.) is native to the Middle East and western Asia. Figs were first domesticated around 11,400 years ago in the lower Jordan Valley (Kislev et al. 2006). Fig fruit are widely consumed, and fig trees are often used as ornamental plants. Figs are grown worldwide with production surpassing 1 billion tons. The five largest fig fruit producers in the world are Turkey (310,000 tons), Egypt (225,295 tons), Morocco (153,472 tons), Iran (130,328 tons), and Algeria (114,092 tons) (FAO 2019). The United States ranks as the eighth largest fig producer in the world, at approximately 28,300 tons; the majority of U.S. production occurs in California (Fig. 1). Figs are commercially propagated by grafting or self-rooted cuttings; these methods favor the dissemination of various pests and diseases, including the viruses and viroids associated with fig mosaic disease (FMD).

FMD is a major disease affecting fig trees throughout the world. It was first described in California in the early 1930s (Condit 1933). Symptoms include chlorotic and yellowish spots, discoloration, deformation, and mosaic patterns on the leaves and fruit (Figs. 2 and 3). Although symptoms have been observed in fig trees for almost a century, the etiological agents associated with FMD have been investigated only within the past decade (Elbeaino et al. 2006, 2007b, 2009a, 2010). Twelve viruses and three viroids have been identified and found to be associated with FMD in various fig producing areas of the world (Table 1).

Most of the viruses associated with FMD are transmitted via vegetative propagation of infected plant material. Exceptions are fig latent virus-1 (FLV) and fig cryptic virus (FCV), which are seed transmissible (Castellano et al. 2009; Fauquet et al. 2005). Vectors

of the viruses and viroids associated with FMD include a mite, *Aceria ficus* (Flock 1955), which has been confirmed to transmit FMV, the causative agent of FMD (Elbeaino et al. 2009a). The transmission rate of FMV can be as high as 70% (Caglayan et al. 2012).

FMD's spatial dispersion is largely due to the use of infected propagative plant material (Minafra et al. 2017). The main form of disease control is prevention, which relies on the production of confirmed pathogen-free plant material. Fig seedlings should be tested to ensure that the plants are virus-free at the time of purchase, before transplantation. An up-to-date list of the primers used for the diagnosis of the viruses and viroids associated with FMD is compiled in Table 2.

The major differences between animal viruses, plant viruses, and viroids are highlighted in Box 1.

Viruses and Viroids Associated with Fig Mosaic Disease

Fig leaf mottle-associated virus 1 (FLMaV-1). Fig leaf mottle-associated virus 1 (FLMaV-1) is a (+)ssRNA virus belonging to the genus *Closterovirus*, in the family *Closteroviridae*. It has filamentous particles 1,800 nm in length, a genome that is approximately 19 kb in length, and it is associated with FMD (Elbeaino et al. 2006) (Fig. 4). The whole genome of FLMaV-1 has not been sequenced yet, which makes phylogenetic studies difficult to conduct. Two open reading frames (ORFs) have been described on the FLMaV-1 genome. One is a partial ORF of a putative polyprotein, which contains a viral helicase 1 domain (pfam01443) (accession number AM279676.1) involved in viral RNA replication. The second ORF is the 70 kDa heat shock protein (HSP70) that contains the conserved protein domain family called the nucleotide-binding domain of the sugar kinase/HSP70/actin superfamily (accession number cl17037) (Fig. 4).

The molecular marker for identification of FLMaV-1 is the HSP70 ORF for which a 351 bp amplicon is amplified by RT-PCR. The amplified region contains the phosphate motifs 1 and 2. A high amino acid residue conservation was found in this genomic region when the sequence of multiple isolates of FLMaV-1 were compared (Elbeaino et al. 2006) (Fig. 5).

FLMaV-1 is vectored by the wax scale insect (*Ceroplastes rusci* L.) (Yorganci and Açikgöz 2019). Several epidemiological studies conducted in various locations where figs are planted commercially

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confirmed that plants displaying FMD symptoms have a high rate of FLMaV-1 infection. In Apulia (southern Italy) and in Tunisia, 64.9% and 28.8% of the samples tested positive for FLMaV-1, respectively (Elbeaino et al. 2006; Nahdi et al. 2006). A survey conducted during the spring of 2016 found that 51% ($n = 49$) of the samples collected in Bosnia and Herzegovina were positive for FLMaV-1, while in Montenegro 57% ($n = 35$) of tested samples were positive (Delić et al. 2017). In China, 5.6% ($n = 252$) of the samples tested were positive

for FLMaV-1 (Mijit et al. 2017). Globally, a literature search concluded that 22% ($n = 435$) of the 2,022 samples of symptomatic and asymptomatic fig plants tested positive for FLMaV-1 (Fig. 1). However, studies need to be conducted to investigate the genetic makeup of the virus, sequence its whole genome, elucidate its impact on fig production worldwide, and establish efficient virus control strategies.

Fig leaf mottle-associated virus 2 (FLMaV-2). Fig leaf mottle-associated virus 2 (FLMaV-2) belongs to the genus *Ampelovirus* in the family *Closteroviridae*. Its virions are approximately 2,100 nm in length and 12 nm in diameter with a (+)ssRNA genome of approximately 19 kb in size (Elbeaino et al. 2007a). Six ORFs have been described in the FLMaV-2 genome, but the full genome has yet to be sequenced (Fig. 6). The first ORF codes for a putative coat protein duplicate containing a conserved domain from a Closter coat superfamily (accession number cl03354), which is present in several members of the family *Closteroviridae*. BLAST analysis of the first ORF sequence revealed no FLMAV-2 homologs, with the closest alignment having 41% identity with the coat protein of little cherry virus 2. The second ORF has no putative function or conserved domains. The third ORF codes for an HSP70 protein with an HSP70_NBD domain (accession number cd10170). The fourth ORF contains a P55 protein and a viral_Hsp90 domain (accession number cl20248). The fifth ORF, p22, has no putative function or domain discovered yet. The sixth ORF is predicted to be the coat protein, with the conserved domain Closter coat (accession number cl03354). There are currently sequences from 28 isolates available in NCBI and the majority of the sequences are from the HSP70 gene, the molecular marker for the identification of FLMaV-2. The sequence of isolate FAR-1 (MG4075556.1) was submitted as a variant of FLMaV-2; however, based on a 97% sequence similarity to FLMaV-1 isolates, it is most likely a member of the genus *Closterovirus* rather than the genus *Ampelovirus*.

Surveys of FLMaV-2 indicate geographic variation in the occurrence and spread of this virus. In Montenegro, no positive samples ($n = 35$) were observed, whereas in Bosnia and Herzegovina, only 2% of the samples ($n = 49$) tested positive for FLMaV-2 (Delić et al. 2017). In Lebanon, the occurrence of this virus was up to 24.9% ($n = 102$) in fig plantations (Elbeaino et al. 2007a), and 31.1% in Syria, (Elbeaino et al. 2012). In Turkey, only 4.5% of the samples ($n = 132$) tested were positive for FLMaV-2 (Caglar et al. 2011). Globally, approximately 2,257 samples were tested for the presence of FLMaV-2 with 202 positive samples (9%) (Fig. 7). The occurrence and distribution of FLMaV-2 have been studied primarily in the Mediterranean region, which begs the question of the presence of this virus in other fig producing areas of the world.

Fig leaf mottle-associated virus 3 (FLMaV-3). Fig leaf mottle-associated virus 3 (FLMaV-3) is a putative member of the genus *Closterovirus*, in the family *Closteroviridae* and is associated with

Fig. 1. Fig plantations. **A**, A fig plantation in California in the early 1900s. **B**, A modern fig plantation in California.

Fig. 2. An asymptomatic leaf of *Ficus carica* (top left), and leaves displaying symptoms of fig mosaic disease (top right, bottom left, bottom right).

Fig. 3. Fruits collected from a fig plant displaying fig mosaic disease symptoms.

FMD (Elçi et al. 2012; Norozian et al. 2014). FLMaV-3 has a linear (+)ssRNA genome; however, its whole genome has not been sequenced yet (Fig. 8), and there is currently no prediction on genome size. Only four partial sequences are available in GenBank from the heat shock protein 70-like gene (HSP70h): one sequence from the U.S.A. (EF654103.1) (Tzanetakis et al. 2010) and three sequences from Iran (MG407557.1, KM516761.1, and KM516760.1) (Norozian et al. 2014). Each of the HSP70h proteins contains an NBD sugar kinase HSP70 actin domain, which is highly conserved among the four isolates (Fig. 9).

A survey conducted in Iran found only one positive sample out of 20 tested (Norozian et al. 2014). Globally, testing for FLMaV-3 has been extremely limited ($n = 30$) with only one positive sample (3%). Further studies are essential to sequence and annotate the full genome of FLMaV-3, identify vectors, determine global spread, and gauge the severity of damage caused by FLMaV-3.

Arkansas fig closterovirus-1 (AFCV-1) and Arkansas fig closterovirus-2 (AFCV-2). Two new viruses, Arkansas fig closterovirus-1 (AFCV-1) and Arkansas fig closterovirus-2 (AFCV-2), were found to be associated with FMD. They were first reported in 2008 from the state of Arkansas, U.S.A. (Tzanetakis et al. 2010). AFCV-1 and AFCV-2 are (+)ssRNA viruses that have tentatively been proposed to belong to the genus *Closterovirus* and their occurrence has been reported only in the U.S.A. The isolates of AFCV-1 remain unverified in GenBank and there are currently no sequences available for AFCV-2, although diagnostic primers are available (Tzanetakis et al. 2010).

BLAST analysis of the sequence of AFCV-1 isolate YN4 (JN882590.1) shows 97.39%, 95.42%, 95.42%, and 90.01% sequence identity to four FLMaV-3 isolates (accession numbers: EF654103.1,

MG407557.1, KM516761.1, and KM516760.1) (Fig. 10). There are no conserved domain calls for any of the AFCV-1 isolates, or for the aligned region of FLMaV-3 isolates (Fig. 10). Furthermore, after carefully checking the detection primers (AFCV-1F: 5'-CTGTAATCTGT CACCTTCGGG-3' and AFCVIR: 5'-ATGCTTCCTCGGCTGC-3') for the putative AFCV-1, we found that they align on the HSP70h region of the four FLMaV-3 isolate sequences available in NCBI. One wonders if AFCV-1 is actually FLMaV-3 and if AFCV-2 could be a putative variant of FLMaV-3 instead of AFCV-1, although the latter hypothesis is difficult to confirm since no sequences of AFCV-2 are available in NCBI. Without more quantitative and qualitative data, it is difficult to be sure of the true nature of AFCV-1 and AFCV-2; however, we hypothesize that they are members of FLMaV-3. Therefore, we omitted AFCV-1 and AFCV-2 from the global study due to a lack of information on these two viruses.

More studies need to be conducted to corroborate the hypothesis that AFCV-1 is actually FLMaV-3. Also, if AFCV-1 and AFCV-2 are proved to be independent species, it is unlikely that these viruses occur only in the United States. Currently, only one study has identified AFCV-1 and AFCV-2 (Tzanetakis et al. 2010) and it is imperative to perform surveys in other fig producing countries to acquire more epidemiological and biological information on these viruses. This information would contribute to the development of possible control methods, document the geographic range of the viruses, confirm pathogenicity, and quantify the economic losses they cause.

Fig mosaic emaravirus virus (FMV). Fig mosaic emaravirus virus (FMV), also known as fig mosaic virus, is a (-)ssRNA virus and a member of the *Emaravirus* genus, in the family *Fimoviridae*. The genome is multipartite (contains six RNA segments). Each RNA segment has a single ORF with a 5' and a 3' end, and each

Table 1. Viruses and viroids associated with fig mosaic disease in fig trees

Virus/viroid species	Genus	Countries	References
<i>Fig leaf mottle-associated virus 1</i> (FLMaV-1)	<i>Closterovirus</i>	Syria, Saudi Arabi, Egypt, Montenegro, Lebanon, Tunisia, Italy, Albania, China	Alhudaib 2012; Elbeaino et al. 2006, 2007b, 2009b, 2012; Elbeshehy and Elbeaino 2011; Mijit et al. 2017; Nahdi et al. 2006; Perović et al. 2016
<i>Fig leaf mottle-associated virus 2</i> (FLMaV-2)	<i>Ampelovirus</i>	Syria, Egypt, Lebanon, Tunisia, Albania, Algeria, Turkey	Caglar et al. 2011; Elbeaino et al. 2007a, 2009b, 2012; Elbeshehy and Elbeaino 2011
<i>Fig leaf mottle-associated virus 3</i> (FLMaV-3)	<i>Closterovirus</i>	Iran	Norozian et al. 2014
<i>Arkansas fig closterovirus-1</i> (AFCV-1)	<i>Closterovirus</i>	United States	Tzanetakis et al. 2010
<i>Arkansas fig closterovirus-2</i> (AFCV-2)	<i>Closterovirus</i>	United States	Tzanetakis et al. 2010
<i>Fig mosaic virus</i> (FMV)	<i>Emaravirus</i>	Syria, Iran, Saudi Arabi, Egypt, Montenegro, Japan, China, Turkey	Ale-Agha and Rakhshandehroo 2014; Alhudaib 2012; Caglar et al. 2011; Elbeaino et al. 2012; Elbeshehy and Elbeaino 2011; Ishikawa et al. 2012; Mijit et al. 2015, 2017; Perović et al. 2016; Tzanetakis et al. 2010
<i>Fig latent virus 1</i> (FLV-1)	<i>Trichovirus</i>	United States	El-Air et al. 2013; Elbeaino et al. 2012; Elbeshehy et al. 2017
<i>Fig mild mottle-associated virus</i> (FMMaV)	<i>Closterovirus</i>	Syria, Tunisia, Saudi Arabia	Alishiri et al. 2018a; El-Air et al. 2013; Elbeaino et al. 2012; Elbeshehy and Elbeaino 2011; Mijit et al. 2017
<i>Fig cryptic virus</i> (FCV)	<i>Alphacryptovirus</i>	Albania, Algeria, Italy, Lebanon, Syria, Tunisia, Iran, Turkey	Ale-Agha and Rakhshandehroo 2014; Elbeaino et al. 2011b; Elçi et al. 2017
<i>Fig fleck-associated virus</i> (FFKaV)	<i>Maculavirus</i>	Albania, Algeria, Italy, China	Ale-Agha and Rakhshandehroo 2014; Elbeaino et al. 2011a, 2012; Elçi et al. 2017; Mijit et al. 2017
<i>Fig badnavirus 1</i> (FBV-1)	<i>Badnavirus</i>	Lebanon, Syria, Tunisia, Iran, Turkey	Alimoradian et al. 2014; Alishiri et al. 2018b; Laney et al. 2012; Mijit et al. 2017; Tzanetakis et al. 2010; Vončina et al. 2015
<i>Apple dimple fruit viroid</i> (ADFVd)	<i>Apscaviroid</i>	United States, Iran, Croatia, China	Chiumenti et al. 2014
<i>Citrus exocortis viroid</i> (CEVd)	<i>Pospiviroid</i>	Italy	Yakoubi et al. 2007
<i>Hop stunt viroid</i> (HSVd)	<i>Hostuviroid</i>	Tunisia	Elbeaino et al. 2012; Yakoubi et al. 2007
		Tunisia, Syria	

is individually encapsulated in a separate virus capsid (Walia et al. 2014) (Fig. 11). RNA1 is 7,039 bp long, and codes for the RNA dependent RNA polymerase (RdRp). This ORF is used as a molecular marker for diagnoses by RT-PCR and for phylogenetic

BOX 1

Animal viruses, plant viruses, and viroids. A typical animal virus is composed of nucleic acid (DNA or RNA) that is protected by a protein-based hard shell (the capsid), which is surrounded by an external membrane (the envelope) composed of phospholipids, proteins, and glycoproteins. Plant viruses generally do not have this envelope; the nucleic acid (DNA or RNA) is protected by the capsid. Viroids are RNA molecules lacking a capsid and an envelope. Viroid genomes are approximately 10 times smaller than those of a classic plant virus. There are a few examples of animal viruses lacking envelopes and plant viruses containing envelopes; however, to date all the viroids discovered are composed of “naked” RNA.

studies. There are five conserved motifs (A–E) used for virus identification toward the 3′ end of the genome. RNA2 is 2,252 bp in length and codes for a putative glycoprotein. RNA3 is approximately 1,490 bp long that putatively codes for the nucleocapsid protein (NP). RNA4 is approximately 1,452 bp long that presumably codes for a movement protein as it contains an emaravirus P4 movement domain (accession number c124917). No information is available on the functions or the proteins coded by RNA5 (1,752 bp) and RNA6 (1,212 bp).

We used Illumina high throughput technology (HTS) to sequence the total RNA from an FMD symptomatic fig leaf sample isolated from a Connecticut orchard (Fig. 2). The ribosome RNA depletion method was used for library preparation (NEB, Ipswich, MA, U.S.A.). A consensus whole genome sequence of FMV isolate CAES was extracted from the HTS dataset (150 bp reads) using established bioinformatics tools (Villamor et al. 2019). The CAES isolate contains the six (–)ssRNA segments. BLAST analysis of the RNA-dependent RNA-polymerase CAES sequence showed >97%, 94.92%, and 92.17% nucleotide identity with 100% query cover to other FMV isolates deposited in GenBank.

FMV is transmitted by grafting and vectored by an eriophyid mite, *Aceria ficus* (Caglayan et al. 2012; Flock 1955). Among all viruses associated with fig mosaic disease, FMV is the only virus identified to be an etiological agent of FMD (Elbeaino et al. 2009a). Recently, antibodies were developed against the nucleocapsid protein (NP) of FMV (Shahmirzaie et al. 2019), an important step on the development of an immunoassay for a rapid and cost-efficient diagnosis of this virus. Furthermore, FMV was found naturally infecting Persian cyclamen (*Cyclamen persicum* Mill.) (Elbeaino et al. 2018), which is the only non-fig host found for this virus, adding more complexity to the management of this important fig virus.

Surveys indicated that FMV is one of the most commonly found viruses infecting fig plants in the world. However, the percentage of

Table 2. RT-PCR primers used to identify viruses and viroids associated with FMD

Virus/viroid	Primer	Sequence	Gene	Reference
FLMaV-1	CPtr-a	5′-CGTGGCTGATGCAAAGTTTA-3′	HSP70h	Elbeaino et al. 2007a
	N17-s	5′-GTTAACGCATGCTTCCATGA-3′		
FLMaV-2	F3-s	5′-GAACAGTGCCTATCAGTTT	HSP70h	Elbeaino et al. 2007a
	F3-a	5′-TCCCACCTCCTGCGAAG		
		CTAGAGAA-3′		
FLMaV-3	FLMaV-3sF	5′-CTGTATCTGTCTATTACCTCTTCGGG-3′	HSP70h	Norozian et al. 2014
	FLMaV-3sR	5′-CTGTATCTGTCTATTACCTCTTCGGG-3′		
FMV	EMARV-GP-s	5′-GGGTACATATGCGTCATTCTTG-3′	RdRp	Walia et al. 2009
	EMARV-GP-a	5′-CGTTTGTCTTGGATCACAGCAA-3′		
FLV-1	FLV1-s	5′-CCATCTTCAACACACAAATGTC-3′	Coat protein	El-Air et al. 2013
	FLV1-a	5′-CAATCTTCTTGGCCTCCATAAG-3′		
AFCV-1	AFCV1F	5′-CTGTATCTGTCTATTACCTCTTCGGG-3′	—	Tzanetakis et al. 2010
	AFCV1R	5′-ATGCTTCTCTCGGCTGC-3′		
AFCV-2	AFCV2F	5′-GTTTCGGAATTAGTTAATAVTA-3′	—	Tzanetakis et al. 2010
	AFCV2R	5′-ACCCGCTAGAGTAATCAGTC		
		AAAGTT-3′		
FMMaV	LM3s	5′-AAGGGGAATCTACAAGGGTCG-3′	HSP70h	Elbeaino et al. 2010
	LM3a	5′-TATTACGCGCTTGAGGATTGC-3′		
FCV	FCV-R2s	5′-TTGGCCGACTACTCAAGTCA-3′	CP	Elbeaino et al. 2011b
	FCV-R2a	5′-TGGAGGTAGCATGTGTAGC-3′		
FFKaV	D8-s	5′-TCAATCCCAAGGAGGTGAAG-3′	RdRp	Elbeaino et al. 2012
	D8-a	5′-ACACGGTCAATGAGGGAGTC-3′		
FBV-1	1094F	5′-ACCAGACGGAGGGAAGAAAT-3′	Polypotein	Laney et al. 2012; Tzanetakis et al. 2010
	1567R	5′-TCCTTGCCATCGGTTATCTC-3′		
ADFVd	ADFVd-for	5′-CCCCCTGCGCTACTGATAAAAG-3′	—	Chiumenti et al. 2014
	ADFVd-rev	5′-GTGTTTACCTGGAG		
		GCTCCACTC-3′		
CEVd	CEVd-F	5′-GGAAACCTGGAGGAAGTCGAGG-3′	—	Yakoubi et al. 2007
	CEVd-R	5′-CCGCCTCTTTTCTTTTCCT		
		GCCTGC-3′		
HSVd	78P	5′-AACC CGGGGCAACTCTTCTC-3′	—	Elbeaino et al. 2012
	83M	5′-AACC CGGGGCTCCTTCTCA-3′		

FMV infected plants varies drastically by region: 43% ($n = 21$) in Montenegro (Perović et al. 2016), 56.7% ($n = 90$) in Syria (Elbeaino et al. 2012), 87% ($n = 30$) in Croatia (Vončina et al. 2015), and 7.6% ($n = 132$) in Turkey (Caglar et al. 2011). In China, 44.4% ($n = 252$) of plants tested positive for FMV (Mijit et al. 2017). Globally, approximately 1,924 samples have been tested for FMV and 627 (33%) tested positive for FMV. Future studies should investigate global genome diversity of FMV isolates, vector transmission, and possible management strategies.

Fig latent virus 1 (FLV-1). Fig latent virus 1 (FLV-1) is a (+) ssRNA virus with 700 nm particles and an 8 kb genome composed of four ORFs. It is a member of the genus *Trichovirus* in the family *Betaflexiviridae* (Elbeshehy et al. 2017; Gattoni et al. 2009) (Fig. 12). The whole genome of FLV-1 is yet to be sequenced, but there are four partial genome sequences available in NCBI (accession numbers: KM156763.1, KM156762.1, FN377573.1, and MG407553.1). ORF1 is approximately 4,264 bp in length and it harbors three domains: a 20 G Fe II Oxygenase (accession number pfam03171), a viral helicase 1 with NTPase activity (accession number cl26263), and an RdRp 2 superfamily (accession number cl03049). ORF2 is approximately 1,144 bp and codes for a putative movement protein as it contains a viral movement protein domain (accession number cl03100). According to NCBI's conserved domain search tool, ORF2's protein domain is critical in the early stages of plant virus infection. ORF3 is approximately 1,236 bp in length and codes for the coat protein, which is the molecular marker used for FLV-1 identification. It contains the domain superfamily Tricho coat (accession number cl05455). ORF4 is approximately 348 bp in length and codes for a putative nucleic acid-binding protein. It harbors the domain Carla C4 that includes a motif for a C-4 zinc finger with four conserved cysteine residues (accession number cl03285).

FLV-1 has an unusually high seed transmission rate (92%) in comparison with other seed-transmitted plant viruses, which usually have

a transmission rate of less than 10% (Castellano et al. 2009). Surveys in fig plantations in Syria revealed that 11.1% ($n = 90$) of tested plants were infected by FLV-1 (Elbeaino et al. 2012). In Tunisia, 44% ($n = 85$) of the samples tested positive for FLV-1 (El-Air et al. 2013), while in Saudi Arabia, the percentage was >33% ($n = 60$) (Elbeshehy et al. 2017). Globally, approximately 1,049 samples have been tested for FLV-1 with 168 positive samples (16%). It is important to note that this virus has also been detected in FMD-asymptomatic plants. Disease management should consider testing of asymptomatic plants as they could be overlooked as source of inoculum for this virus.

Fig mild mottle-associated virus (FMMAV). Fig mild mottle-associated virus (FMMAV) is a (+)ssRNA virus and a putative member of the genus *Closterovirus* in the family *Closteroviridae* (Elbeaino et al. 2010). Only 26 partial sequences of FMMAV have been deposited in GenBank, encompassing seven ORFs (accession number FJ611959) (Fig. 13). The full-length genome size has yet to be deciphered and the 5' and the 3' ends have not been sequenced yet. ORF1 codes for a putative RdRp, with a domain for an RdRp 2 superfamily (pfam cl03049). No putative function or domain calls have been found for ORF2 and ORF3. ORF4 codes for an HSP70 protein with an NDB sugar kinase domain (pfam cd10170). ORF5 codes for a p64 protein with a viral Hsp90 domain (accession number cl20248). ORF6 codes for a putative minor coat protein (CPm), and ORF7 codes for a putative coat protein (CP); they both contain the Closter coat domain (pfam cl03354).

Surveys of the fig germplasm collection from the High Agronomic Institute (ISA) in Tunisia revealed that 17.25% ($n = 29$) of the tested plants were infected with FMMAV (Bayoudh et al. 2017). In Iran, 11% of the samples tested ($n = 44$) were positive for FMMAV (Alishiri et al. 2018a); in Syria, 12.2% of the samples tested ($n = 90$) were positive (Elbeaino et al. 2012); and 10% in Montenegro ($n = 21$) (Perović

Fig. 4. The partial FLMAV-1 genome map from the sequences available in NCBI with the predicted conserved functional units.

Fig. 6. Partial FLMAV-2 genome map from the most complete partial sequence in NCBI (FJ473383.1) with the predicted conserved functional units.

Fig. 5. The amino acid sequence logo of the FLMAV-1 identification molecular marker, a 351 bp region of the HSP70 gene. The sequence logo displays high conservation among the 10 FLMAV-1 isolates with 100% query over from NCBI used in the analysis (accession numbers: ANB45579.1, ANB45584.1, ANB45588.1, ANB45587.1, ANB45581.1, ANB45583.1, CTQ47930.1, ANB45585.1, ANB45582.1, and ANB45580.1).

et al. 2016). The incidence of FMMAV was relatively low in China, where only 0.4% of the samples were positive ($n = 252$) (Mijit et al. 2017). Globally, approximately 1,548 samples have been tested and 140 of them (9%) were positive for FMMAV. Furthermore, studies on the occurrence of FMMAV have been limited to only areas where FMD has been reported.

Fig cryptic virus (FCV). Fig cryptic virus (FCV) is a dsRNA bipartite genome virus. The RNA1 segment is approximately 1,696 bp and codes for the RdRp protein with an RT-like domain (accession number cl02808). The RNA2 segment is approximately 1,415 bp and codes for the coat protein (CP) (Elçi et al. 2017) (Fig. 14). FCV is the first member of the genus *Alphacryptovirus*, in the family *Partitiviridae*, to be detected in fig trees; it was first reported in fig trees in Italy in 2011. Although FCV is associated with FMD, it does not induce symptoms in fig trees, and it is neither graft nor mechanically transmitted (Elbeaino et al. 2011b).

A survey conducted in the Mediterranean region revealed that 18.5% of the samples tested ($n = 210$) were positive for FCV (Elbeaino et al. 2011b). The highest occurrence of FCV was reported in Lebanon at 23.3% ($n = 60$). In Turkey, 20% of the tested plants ($n = 65$) were positive for FCV (Elçi et al. 2017), while in Iran, the incidence was only 4.5% ($n = 197$) (Ale-Agha and Rakhshandehroo 2014). FCV does not appear to induce noticeable symptoms in fig plants. However, all plant material should be tested for FCV before

planting to avoid the introduction of this virus in newly planted areas.

Fig fleck-associated virus (FFKaV). Fig fleck-associated virus (FFKaV) is a (+)ssRNA virus, and is a member of the genus *Maculavirus* in the family *Tymoviridae* (Fig. 15). It was first reported in Apulia (southern Italy) in 2011 (Elbeaino et al. 2011a).

Surveys conducted in Albania, Algeria, Italy, Lebanon, Syria, and Tunisia revealed that 20% of the samples ($n = 210$) tested positive for FFKaV (Elbeaino et al. 2011a). In Syria, 36.7% ($n = 90$) collected in nine different cities were positive for FFKaV (Elbeaino et al. 2012). The occurrence of this virus was much lower in Turkey, where 9.2% ($n = 65$) of the samples were positive for FFKaV (Elçi et al. 2017). Similarly, in Iran, 8.6% tested positive ($n = 197$) (Ale-Agha and Rakhshandehroo 2014). In contrast, a relatively high rate of infection was found in China, with 44% of samples ($n = 252$) positive for

Fig. 8. Partial FLMaV-3 genome map drawn from the sequences available in NCBI with the predicted conserved functional unit.

Fig. 7. Global distribution of the viruses and viroids associated with fig mosaic disease. Each circular chart depicts the associated pathogen with its percentage of infected samples from the total number of samples (n) tested.

FFKaV (Mijit et al. 2017). Globally, approximately 1,286 published samples have been tested for FFKaV, with 250 (19%) positive samples detected.

Blast searches of the NCBI database revealed that a variant of this virus was discovered in China, named fig fleck-associated virus 2 (accession numbers: KT438719.1, KT438721.1, KT438722.1, KT438723.1, and KT438724.1); however, no description of this virus was found in the literature. Therefore, more research is needed to describe this virus further and determine if it is indeed a new species or a new strain of FFKaV. Furthermore, there is a need to verify if these viruses are associated with FMD and to what extent, if any, they can be detrimental to fig production.

Fig badnavirus 1 (FBV-1). Fig badnavirus 1 (FBV-1) belongs to the genus *Badnavirus*, family *Caulimoviridae*; it is a dsDNA virus with a genome of 7,141 bp. The genome is monopartite, open circular, and harbors three ORFs with a 3 bp overlap on each reading frame (Fig. 16). Eight complete genome sequences for different FBV-1 isolates are available in GenBank. One of these isolates (accession number MK348055.1) was called to have a putative ORF4

(Alishiri et al. 2018b). Accordingly, the putative ORF4 was found in the one of the FBV-1 isolates in GenBank. However, in GenBank, the genomes of all the other seven isolates cited in the paper were published without the putative ORF4. To shed light on this discrepancy, we extracted total RNA from an FMD symptomatic fig plant leaf collected in a Connecticut vineyard in October 2019 and processed it for high throughput sequencing (HTS) via Illumina platform using the ribosome RNA depletion library preparation method (NEB, Ipswich, MA, U.S.A.). A consensus whole genome sequence of FBV-1 was extracted from the HTS dataset (150 bp reads) using well-established bioinformatics tools (Villamor et al. 2019). The CAES isolate was 7,141 bp long and contained a short 5' end of 217 bp and a longer 3' end of 579 bp. BLAST analysis of the CAES sequence showed over 99% nucleotide identity to the other eight isolate sequences available in the NCBI database (Fig. 16). Only three ORFs were detected on the FBV-1_CAES genome after running Genemark S, a gene prediction algorithm. The same analyses were also run on the genomes from the other eight FBV-1 isolates, and only three ORFs were detected on those genomes. Based on all this

Fig. 9. A sequence logo of the partial HSP70h protein from the four FLMaV-3 isolates available in GenBank (accession numbers: EF654103.1, MG407557.1, KM516761.1, and KM516760.1). The sequence logo shows high amino acid conservation among the isolates.

Fig. 10. Depiction of the partial genome of AFCV-1 compared with the genome of FLMaV-3 isolates available in NCBI.

evidence and the extremely conserved nature of the FBV-1 genome (Fig. 16), it is likely that ORF4 does not exist, but it may be part of the large ORF3 polyprotein. We conclude that the ORF4 calling was an error caused by mis-annotation and that the FBV-1 genome contains only three ORFs, comparable to most badnaviruses species (Bhat et al. 2016).

FBV-1 was first reported in 2008 in the U.S.A. (Tzanetakis et al. 2010). It was later demonstrated that, besides replicating, FBV-1 integrates into the fig genome. It is found infecting both asymptomatic and symptomatic fig trees (Laney et al. 2012). FBV-1 is widespread in fig producing regions and it was found to be prevalent in the National Germplasm Repository (NCGR) in Davis, California, U.S.A. (Laney et al. 2012). In Croatia, 30 samples were tested and all were positive for FBV-1 (Vončina et al. 2015). In Iran, 66.6% of samples tested positive ($n = 92$) (Alimoradian et al. 2016). In China, 48% of fig samples ($n = 525$) assayed were positive for FBV-1 (Mijit et al. 2017). Although widespread, globally only 773 fig samples have been tested for FBV-1 with 454 (59%) positive for the virus.

FBV-1 is one of the most common viruses found in fig trees because it integrates the fig genome and it can be detected in asymptomatic fig trees. This raises the question of whether FBV-1 causes symptoms of FMD or if it is just coincidentally found in plants displaying FMD. More research needs to be conducted to address this point and to develop ways of generating fig planting material that is free of FBV-1, as it was detected in every commercially available fig plant, meristem tip cultured plants, and fig seedlings tested in the U.S.A. (Laney et al. 2012).

Apple dimple fruit viroid (ADFVd). Apple dimple fruit viroid (ADFVd), genus *Apscaviroid*, family *Pospiviroidae*, is a viroid. Viroids are the smallest plant disease-causing agents. They have single-stranded, circular, and non-protein-coding RNA pathogens of only 246 to 401 nucleotides in size (Navarro et al. 2012). ADFVd is 306 nucleotides long; it was first identified in the Starking Delicious apple cultivar in Italy in 1996 (Di Serio et al. 1996). Then in 2012, also in Italy, it was found in FMD symptomatic fig trees (Chiumenti et al. 2014). Viroids are known to occur at extremely low titer (number of infectious units per amount of sample) in their hosts (Navarro et al. 2012), which often results in false negative RT-PCR results. Therefore, high throughput sequencing (HTS) to characterize viroid-derived small RNAs of 21 to 24 nt (vd-sRNAs) has been established as a powerful tool for the detection and characterization of viroids. Even though ADFVd was detected in fig trees displaying FMD symptoms (Chiumenti et al. 2014), it accumulates at low levels in figs, and more research is needed to elucidate the role of this viroid in causing disease in fig plants. Other FMD associated viruses (FMV,

Fig. 12. Partial FLV-1 genome depicting the four gene sequences available in NCBI (accession numbers: KM156763.1, KM156762.1, FN377573.1, and MG407553.1).

Fig. 13. FMMAV genome map containing the largest partial sequence available in NCBI (FJ611959).

Fig. 14. Complete FCV genome map containing the reference sequences NC_015494 and NC_015495, dsRNA-1 and dsRNA-2, respectively.

Fig. 15. An FFKaV genome map from NCBI sequences and from Elbeaino et al. (2011a).

FMV segmented genome

Fig. 11. The complete FMV genome map containing six RNA segments. The predicted conserved functional units for RNA1 and RNA4 are highlighted inside the boxes depicting the segments.

FLV-1, and FBV-1) were also detected in the same samples that were positive for ADFVd (Chiumenti et al. 2014).

Citrus exocortix viroid (CEVd). Citrus exocortix viroid (CEVd) is a member of the genus *Pospiviroid*, in the family *Pospiviroidae*. It was identified as the causal agent of bark shelling symptoms in citrus in the 1940s (Benton et al. 1950). Since then, it has been isolated from several plant hosts (e.g., members of the Solanaceae family, and a range of other vegetable crops) (Szychowski et al. 2005). In 2007, a study in Tunisia reported the first occurrence of CEVd associated with FMD (Yakoubi et al. 2007)—CEVd was detected in every FMD-symptomatic plant tested ($n = 13$), but it was not detected in any of 14 asymptomatic plants tested. The occurrence of CEVd in FMD-symptomatic plants does not guarantee that this viroid causes FMD. Additional studies are necessary to dissect the role of CEVd in the occurrence of FMD.

Hop stunt viroid (HSVd). Hop stunt viroid (HSVd) is a member of the genus *Hostuviroid* in the family *Pospiviroidae* (Astruc et al. 1996; Di Serio et al. 2014). HSVd has the broadest host range known for a viroid, infecting plants such as hop, cucumber, grapevine, citrus, plum, peach, pear, and apricot. In 2007, it was detected in fig trees displaying FMD symptoms in Tunisia (Yakoubi et al. 2007). As for CEVd, all 13 FMD-symptomatic plants tested were positive for HSVd and none of the 14 asymptomatic plants tested positive for this viroid. Furthermore, in Syria, HSVd was identified in 13.3% of sampled trees ($n = 90$) (Elbeaino et al. 2012). Of the three viroids detected in FMD-symptomatic plants, HSVd is the most consistently associated with this disease. Globally, approximately 227 samples have been tested for HSVd, with 37 (16%) testing positive

for this viroid. However, there are no studies on pathogenicity or effects that this viroid may have on fig orchards.

Concluding Remarks and Future Prospects

Fig mosaic disease (FMD) affects fig trees worldwide, causing economic losses and posing a serious threat to fig production. Since its discovery, 12 viruses, including a confirmed causal agent (fig mosaic virus, FMV), and three viroids have been detected in fig trees displaying FMD symptoms (Elbeaino et al. 2009a). More research into the epidemiology of these viruses and viroids is needed to determine whether they are truly associated with FMD, are synergistic with FMV, or are just part of the plant's virome without association of this disease complex. As high throughput sequencing continues to generate viral metagenomic data sets, new viruses and viroids that may not have an etiological association with figs will be uncovered. It is important to perform thorough testing with all the viruses associated with FMD to elucidate disease-causing viruses and target efforts and resources of FMD management toward the viruses and viroids that are causing crop damage.

The genomes of most of the associated viruses/viroids remain to be fully sequenced, with some isolates containing only partial ORFs published in GenBank. While the sequences of most viruses have highly conserved regions that help in making inferences on the genus and species, whole genome sequencing of several isolates of the same species in different areas of the world would be highly beneficial for phylogenetic and mutational analysis studies. Those analyses would help to pinpoint the geographic origin and evolutionary dynamics of those pathogens, thereby supporting and validating

Fig. 16. A comparison of the genomes of the CAES_FBV-1 isolate with the full genome FBV-1 isolates available in NCBI. The purple box depicts the ORF4 called for isolate MK348055.1 that our evidence suggests does not exist but was rather an annotation error.

epidemiological and surveillance methods designed to mitigate the spread of these viruses and viroids in fig plantations.

Combining the data from published FMD studies begins to paint a global picture of the distribution of the viruses and viroids associated with FMD over the last 15 years. From the data collected, FLMaV-2, FLMaV-1, and FMV have been tested most frequently ($n > 1,900$). FLV-1, FMMaV, FFKaV, FCV, and FBV-1 have had between 700 and 1,500 samples tested. However, FLMaV-3, ADFVd, HSVd, and CEVd have had only 30 to 227 samples tested globally. AFCV-1 and AFCV-2 were not included in this portion of the review because AFCV-1 is most likely a variant of FLMaV-3 and not a novel virus infecting fig. Although primers for RT-PCR assays exist, AFCV-2 sequences are not available in the NCBI database. Properly designed epidemiology studies will provide a new understanding of FMD and aim detection efforts in the direction of the viruses and viroids that cause this disease complex.

This review will facilitate future studies of FMD encompassing all viruses and viroids associated with this disease complex. We highlighted herein important areas of future research and emphasized the importance of continued surveys of fig trees and germplasm, as well as the importance of each virus and viroid in disease development, isolate aggressiveness alone or in combination with other FMD-associated viruses, and vectors. Geographic studies of the disease will help determine where the viruses and viroids associated with FMD are located. The development of isolation protocols to study these viruses and viroids in vivo would be beneficial for characterization and symptom development studies with a reproducible protocol. This information would serve to properly conduct disease resistance testing, diagnostic development, and phylogenetic studies.

BOX 2

Virion. An entire virus particle, consisting of a core of nucleic acid (either ribonucleic or deoxyribonucleic acid - RNA or DNA) and a capsid.

Virus titer. The concentration of infectious virus particles in a sample.

Molecular markers. Molecular markers are sequences on a gene of interest that are being targeted. These sequences can be used to identify viruses of importance in molecular assays such as polymerase chain reaction (PCR).

Reading frame. The reading frame refers to one of the three possible ways to read a nucleotide sequence.

Open reading frame (ORF). A portion of the genetic sequence that can be transcribed into amino acids and translated into proteins.

Sequence logo. An amino acid sequence logo depicts the frequency of amino acids in multiple sequences aligned at each position. The height of each letter is an indication of the conservation and frequency of the amino acid.

Illumina high throughput sequencing. A technology that can sequence multiple DNA molecules of a sample in parallel. This sequencing technique facilitates the sequencing of billions of nucleotides in a single run.

Immunoassay. A molecular technique used to measure or identify specific proteins (e.g., viral capsid) through labeled antibodies.

BLAST. The Basic Local Alignment Search Tool is a computational biology tool that is used to input a nucleotide sequence and search for similar sequences in the online databases.

NCBI. The National Center for Biotechnology Information (NCBI) is one of the major online research databases. They develop and maintain molecular and bibliographic databases as a part of the U.S. National Library of Medicine (NLM).

Virome. A group of viruses investigated and described by the sequences of viral nucleic acids found. Some viruses in the virome can be associated with certain host organisms or diseases.

Data from characterization studies will aid the development of control methods, plant breeding, and phylogenetic studies on each virus and viroid associated with FMD. For now, an important step forward in the organization and fine-tuning of FMD is to standardize the names of each associated virus. Plants with FMD in both the Old World and the New World may play a significant role in the expression of the disease. It is possible that some of the newly identified viruses may share vectors that may account, in addition to clonal propagation, for the large number of viruses and viroids that fig harbors.

A glossary of terms appearing in this article appears in Box 2.

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