

SOME ASPECTS REGARDING FIG (*FICUS CARICA L.*) PATHOLOGY IN ROMANIA

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Abstract

The milder winters and longer-than-normal warm periods of recent years in Romania have increased the popularity of the fig trees (*Ficus carica L.*) among fruit growers. Fig trees have proven to be quite resistant to diseases and pests in our continental temperate conditions. However, in recent years, several foliar diseases have been observed. Our research focused on detecting and identifying main fig diseases on the existing germplasm collection of the Faculty of Horticulture, USAMV of Bucharest. Our results highlight the presence of Fig Mosaic Disease (FMD) and brown spot (*Passalora trichophila*) on leaves. Black mold (*Aspergillus niger*) and fruit mold (*Cladosporium sp.*) have been identified on fig fruits. Also, the pathogen *Fusarium oxysporum* has been detected on fig cuttings with symptoms of wilting and basal rot. To our knowledge, this is the first report in Romania of FMD, *Passalora trichophila*, and *Fusarium oxysporum*. Further studies are needed to identify the complex of different viruses responsible for FMD in Romania.

Key words: fig tree, Fig Mosaic Disease, *Fusarium oxysporum*, *Passalora trichophila*.

INTRODUCTION

The fig tree (*Ficus carica L.*) is one of the first cultivated fruit trees (Vidaud et al., 1997; Aljane, 2004), being mentioned in myths and legends (Sinha, 2003), as well as in sacred texts, the Bible, Talmud and Quran (Rahmani and Aldeebasi, 2017; Tomescu, 2014).

The discovery of fossilized figs, dated to 9400 - 9200 BC, suggests that the fig was cultivated at the same time as rice (Asia), but before the cultivation of wheat, barley and legumes (Kislev et al., 2006).

The fig is mentioned in the works of Herodotus, Homer, Aristophanes and Plato, while interesting information about pollination, reproduction and the benefits of fruits on human health can be found in the writing "Research on Plants" by Theophrat.

Historical sources mention that in China, the fig was cultivated, along with lemons and almonds, since 2660 BC (Tomescu, 2014). In Europe, the fig culture has been known since the 8th century. In our country, the fig tree has been brought since Antiquity. Although the origin of the fig tree on Romanian territory is still debated, its appearance being attributed to the Greeks,

Romans, Turks and Armenians, the introduction of figs into the Romanian diet was influenced by the Ottomans (Giurescu, 1966).

Currently, in our country, due to climate change and the dissemination of the benefits brought by the consumption of figs, more and more farmers are thinking of establishing fig orchards. However, fig trees are most often found in private gardens in the country (Stănică, 2017). Research into the origin of the fig tree has placed it in western Asia, the Middle East, and certain areas of the eastern Mediterranean (from eastern Greece to Afghanistan).

Most fig varieties originate from *Ficus carica L.*, a native species from Asia Minor (according to Mihăescu, 1977). Their exact number is, however, unknown due to problems of synonymy and homonymy (Saddoud et al., 2008).

It is considered that, in cultivation, there are over 1,000 fig varieties and biotypes adapted to the pedoclimatic conditions of different areas (Drobotă, 1986).

From a culinary and therapeutic point of view, the fig is a valuable plant. Figs are an important source of minerals and vitamins, lipids, phenols and enzymes (Hoza, 2000; Mitra, 1997),

proteins, amino acids, carbohydrates, but also easily assimilable sugars. The high medicinal value is given by the presence of phytochemicals (Naseer, 2021). Fig leaves also have a number of therapeutic properties and can be used in powder form in various mixtures or infusions.

Fig trees are sensitive to abiotic and biotic stress factors (Mostowfizadeh-Ghalamfarsa et al., 2022), which can affect fruit and leaf production. Among the well-documented and studied fig tree pests *Acaria fici* (fig mite), *Phrynetes spinator* (fig beetle) and the nematodes *Hemicyclophora arenaria*, *Helicotylenchus multicinctus*, *Hoplolaimus* sp. and *Xiphinema* sp. (Faccoli et al., 2016; Kohl, 2011; Luc et al., 2005). The fig woolly aphid (*Ceroplastes rusci*), which feeds on the sap of the tissues, causing a general weakening of the plant, is also frequently reported.

An important viral disease reported worldwide is fig mosaic virus (Norman and Ali, 2013), which affects all fig varieties. Fig mosaic was initially reported in California, Egypt, Australia, and China, and has since spread worldwide through infected planting material and insect vectors. Fig mosaic is caused by numerous viruses, with three associated viroids (Preising, 2021, Jamous et al., 2020, Minafra et al., 2017) (Table 1).

Table 1. Fig viruses and viroids

No.	Virus name	Genus
1	<i>Fig leaf mottle-associated virus 1 (FLMaV-1)</i>	<i>Closterovirus</i>
2	<i>Fig leaf mottle-associated virus 2 (FLMaV-2)</i>	<i>Ampelovirus</i>
3	<i>Fig mosaic virus (FMV)</i>	<i>Emaravirus</i>
4	<i>Fig latent virus 1 (FLV-1)</i>	<i>Trichovirus</i>
5	<i>Arkansas fig closterovirus-1 (AFCV-1)</i>	<i>Closterovirus</i>
6	<i>Arkansas fig closterovirus-2 (AFCV-2)</i>	<i>Closterovirus</i>
7	<i>Fig mild mottle-associated virus (FMMaV)</i>	<i>Closterovirus</i>
8	<i>Fig cryptic virus (FCV)</i>	<i>Alphacryptovirus</i>
9	<i>Fig fleck-associated virus (FFKaV)</i>	<i>Maculavirus</i>
10	<i>Fig badnavirus 1 (FBV-1)</i>	<i>Badnavirus</i>
11	<i>Apple dimple fruit viroid (ADFVd)</i>	<i>Apscavirid</i>
12	<i>Citrus exocortis viroid (CEVd)</i>	<i>Pospiviroid</i>
13	<i>Hop stunt viroid (HSVd)</i>	<i>Hostuvirid</i>

Source: Minafra et al., 2017, Jamous et al., 2020

Fig mosaic was first reported and described in the early 1930s in California by Condit and Horne (1933), and has spread to several fig-growing countries, including Turkey (Ülkümen et al., 1948; Özalp and Heper, 1972). The disease is not transmissible through latex or seeds (Martelli et al., 1993; Elbeaino et al., 2006), with Flock and Wallace reporting in 1955 that the virus is successfully transmitted by the eriophyid mite, *Aceria fici*, a monophagous mite

that feeds on plants in the *Moraceae* family. The last reports of the presence of the mite were in the United States and Greece in 2023 (<https://www.inaturalist.org>).

Elbeaino et al. (2006) suggested that figs harbor a species of closterovirus, for which they proposed the name *Fig leaf mottle-associated virus* (FLMaV). The same closterovirus has also been reported in Tunisia (Nahdi et al., 2006). Recently, another representative of the *Closteroviridae* has been identified, and is called *Fig leaf mottle-associated virus 2* (FLMaV-2).

The disease is transmitted mainly by vegetative propagation (cuttings) and by insect vectors, including the mites *Aceria ficus* and *Tetranychus urticae*.

Symptoms can be visible on leaves and fruits. Yellowish spots are visible on the leaves that contrast with the green tissue. Later, a rusty coloration appears at the edge of the yellow spots, caused by the death of epidermal or subepidermal cells. Leaf deformation, stunted growth, and yellowing of leaves are also present. Yellowing and deformation may occur on the same shoot with normal leaves. Yellow spots similar to those on the leaves, but less obvious, appear on the fruit. Attack on the fruit leads to premature fruit drop or the formation of small, few fruits.

Fungal and bacterial diseases are considered among the most dangerous for cultivated figs (Lin et al., 2018).

Fig leaves are susceptible to attack by fungal and bacterial pathogens, which cause various diseases and spots.

Fungal pathogens are responsible for rust, fusarium wilt, anthracnose and other leaf spots, root rot (Guillaumin et al., 1993; Johnstone and Jones, 1997; Kim et al., 2000; Sreenivasaprasad and Talhinhas, 2005; Droby et al., 2011; Norman and Ali, 2013; Raheb et al., 2014; Al-Sadi et al., 2015; Kumar and Khurana, 2015; Li et al., 2015; Miyake and Nagai, 2017; Lin et al., 2018).

Fungal infections can rapidly degrade fig wood (Lansky and Paavilainen, 2010).

Among the fungal pathogens, *Alternaria alternata*, *Cladosporium cladosporoides*, *Cylindrocladium scoparium* and *Cercospora fici* have been identified.

Bacterial pathogens can cause leaf spots, as *Pseudomonas syringae* pv. *syringae* (Ivanovic et al., 2018, Norman and Ali, 2013). Symptoms of the disease caused by *Pseudomonas syringae* pv. *syringae* appear as necrotic lesions that, within a few days of infection, become larger, coalesce and cover most of the leaf (Ivanovic et al., 2018). Leaf symptoms appear and develop after cold and rainy weather (Ivanovic et al., 2018).

Crown gall, caused by *Agrobacterium tumefaciens*, is considered one of the most important bacterial diseases of fig trees (Turechek, 2012).

Leaf spot caused by *Cercospora fici* is one of the most widespread diseases of figs (Yao et al., 2015). It was first reported in 1918 in Florida (Matz, 1918) and subsequently in Pakistan, Oman, Africa and China (Crous et al., 1991; Al-Sadi et al., 2015; Wu et al., 2015; Nayab and Akhtar, 2016). In the early stages, small, yellowish to brownish spots appear on the leaf. The lesion expands, becoming reddish-brown with dark brown, irregular edges. The attack is present on both leaves and fruits.

In Romania, fig trees have proven to have increased resistance to diseases and pests. However, in isolated cases, diseases that seriously affected the production and lifespan of an orchard have been reported since 1981.

In recent years, the appearance of some leaf diseases has been observed in fig trees in Romania, as well as an alarming increase in the number of cases of fig mosaic, one of the most widespread diseases affecting most fig varieties (Serrano, 2008).

The present study focuses on foliar and fruit diseases of figs in the collection of the Faculty of Horticulture, USAMV of Bucharest.

This study can provide important data regarding fig pathology in Romania, as there is little information related to fig diseases identification.

MATERIALS AND METHODS

The biological material studied was represented by over 60 fig genotypes from Romania, Croatia, Italy, Iraq and England, propagated in the greenhouse and planted in the experimental field of the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest. Over 50 cuttings made

from the Fiorone Rosso genotype and 50 plants from the Galben Mare genotype were also included in this study.

The study was carried out in 2023 and 2024. Monitoring of fig cuttings was carried out during April - September 2023.

Foliar and fruit pathologies were identified through visual observations. For fungal pathogens, a microscopic examination was performed on symptomatic or incubated plant tissues on PDA medium (Potato Dextrose Agar, Scharlau). The incidence (%) of Fig Mosaic Disease was calculated on Galben Mare genotype.

Cuttings fragments from the Fiorone Rosso genotype, with symptoms of wilting and rot were incubated for seven days on PDA medium at 22-24°C. Colonies characteristic of the genus *Fusarium* were detected. Pure cultures of *Fusarium* isolates were grown on PDA in Petri plates and after 7 days, the cultures were observed for shape and size of conidia and chlamydospores.

The *Fusarium* isolate was also identified based on sequencing of ITS rDNA. The sequencing was carried out with the support of the University of La Laguna, Tenerife, within the CIPEV group. The sequence (677 nucleotides, Figure 1) was BLASTed against Genbank database by the National Center for Biotechnology Information (NCBI: <http://www.ncbi.nlm.nih.gov>; accessed on May, 19, 2024) nucleotide database.

Figure 1. BLASTed sequence - *Fusarium oxysporum*

RESULTS AND DISCUSSIONS

Results on foliar and fruit pathology

Results on foliar and fruit pathology, Both in 2023 and 2024, Fig Mosaic Disease (FMD) affected the fig trees included in this study.

The common macroscopic symptoms associated with FMD on leaves were yellow, diffuse spots (mosaic), deformation and chlorotic mottling as well as both combined; necrotic rust-coloured band developed along the border of the mosaic spot as a result of the death of epidermal cells;

leaf deformations, showing weak, asymmetrical growth, yellowing, necrotic spots; leaf wrinkles. Another observation concerned the fact that both leaves with symptoms and normal leaves were observed on the same branch.

On fruits, FMD was associated with yellow or necrotic spots. The range of symptoms observed and the multitude of viruses present suggest that fig mosaic may be caused by the synergistic action of several viruses, in co-infection.

The pathology associated with FMD is shown in Figures 2 and 3.

The frequency of plants from Galben Mare genotype with FMD was 96%, almost all 50 plants observed being affected.

Another fig leaf pathology was the presence of dark brown spots, initially discrete and limited to the main and secondary veins, later spreading over the lamina and coalescing, with finally irregular and more or less necrotic tissues (Figure 4). Microscopic examination revealed morphological characters characteristic of conidia and conidiophores of *Passalora trichophila* and molecular analysis of the isolate is ongoing.

Several species of the genus *Passalora* are involved in fig leaf spots: *Passalora bolleana* (*Pseudocercospora bolleana*), *P. ficina*, *P. trichophila* and *P. urostigmatis* (Singh et al., 2012). *Passalora trichophila* sp. nov. was identified on the leaves of *Ficus mysorensis* (Moraceae) in a subtropical forest in India (Singh et al., 2012).

From the spots developed on the leaves, isolates belonging to *Cladosporium* genus was isolated (Figure 5). *Cladosporium* sp. was observed on fruits, as well as *Aspergillus niger*. While *Cladosporium* on leaves can be a secondary invader or a cause of leaf spot, on fruit it leads to discoloration and spoilage (Figure 6). On green or ripening figs, *Cladosporium* can appear as small, olive-green specks that enlarge into sunken, yellowish-olive lesions. These spots may turn light brown to black.

Aspergillus niger is well known as black mold on fig fruits, causing fruit decay.

Results on fig cuttings pathology

The rooted fig cuttings of the Fiorone Rosso genotype were monitored until September 2023,

when samples were taken for laboratory analysis. The samples were represented by cuttings with symptoms of wilting and rot (Figure 5).

Colonies developed in Petri dishes with incubated cuttings fragments were identified as belonging to *Fusarium* genus, based on macroscopic and microscopic examination (Figure 6). Pure and monospore cultures were obtained for molecular identification. Characterization of the fungal cultures based on morphology and sequencing of ITS rDNA revealed that our isolate belongs to *Fusarium oxysporum* species.

The species *Fusarium oxysporum* is known for the economic damage caused to horticultural crops, the pathogen being difficult to control. The characteristic symptoms are wilting, the presence of yellow areas between the main veins of the leaves and rotting of the roots and the base of the stem.

The pathogen is a known colonizer of soils and various substrates, surviving for many years in them or in plant debris in the form of mycelium or resistant spores - chlamydospores, in the absence of the host plant. It can also be present in soil debris on greenhouse materials, solar panels, irrigation pipes, mechanical equipment and other field materials. *Fusarium* fungi can cause a number of serious plant diseases, with *Fusarium* being one of the most important pathogens (Beccari, 2022; Leslie and Summerell, 2013).

There are few reports in the literature of the presence of *Fusarium* pathogens in fig trees.

In Romania, the disease was reported in 1984 (Drobotă, 1986). Jahén-Rivera et al. (2021) reported the presence of *Fusarium solani*, *Alternaria alternata* and *Pythium ultimum* isolates in rooting fig cuttings that showed slow growth, chlorosis, wilting, fallen leaves and rot on the stems and roots. Plants produced in greenhouses may exhibit stem and root rot, caused by a complex of pathogens from the genera *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora*, favored by a relative humidity of 80%, temperatures of 25°C and high densities (Beccari, 2022; García et al., 2008).

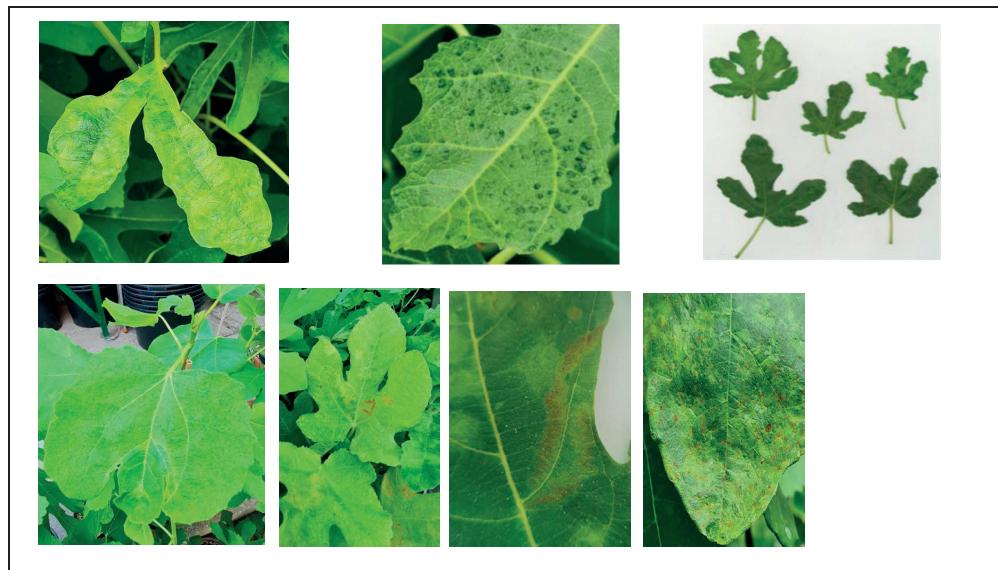


Figure 2. Deformation, embossing, discoloration, necrosis and combined leaf symptoms associated with fig mosaic disease (FMD)



Figure 3. Specific spots of fig mosaic disease (FMD) on leaves and fruits: irregular light green to yellow spots; fruits with yellow spots and necrotic spots



Figure 4. Leaf spotting - characteristic symptoms of *Passalora* spp.



Figure 5. *Cladosporium* spp. on fig leaf



Figure 6. Characteristic symptoms of fruit rot caused by *Cladosporium* spp. and *Aspergillus niger*

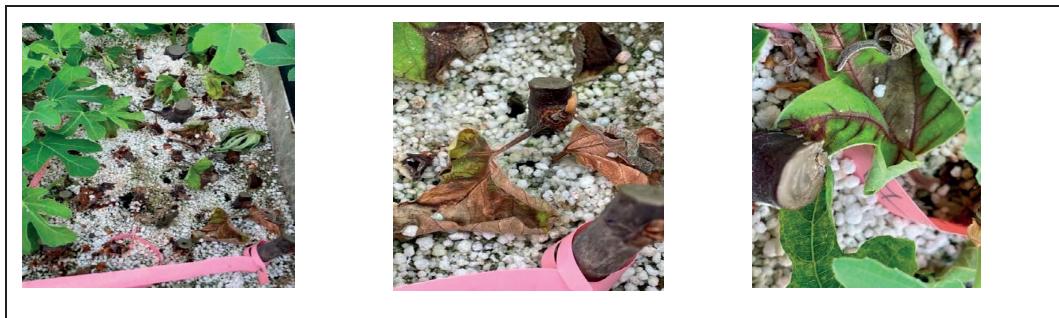


Figure 7. Cuttings with wilt and basal rot symptoms



Figure 8. *Fusarium oxysporum* colonies and conidia

CONCLUSIONS

This study highlights the presence of Fig Mosaic Disease (FMD), *Passalora trichophila* (foliar brown spot), *Cladosporium* spp., and *Fusarium*

oxysporum (wilt and basal rot) as a part of leaves pathology. On fig fruits, FMD, *Cladosporium* spp. and *Aspergillus niger* were detected and identified.

Characteristic symptoms of Fig Mosaic Disease (FMD) were detected on leaves and fruits. FMD is a complex disease associated with mixed virus infections, and different responses in symptom expression due to a specific virus or various virus combinations have been reported. Thus, the prevalence of those viruses remains to be analysed.

Also, another foliar pathogen, *Passalora trichophila* was detected on leaves and was responsible for brown spot disease.

In cuttings with wilting and basal rot symptoms the pathogen *Fusarium oxysporum* was detected and identified through morphological and molecular analysis.

The presence of *Cladosporium* spp. and *Aspergillus niger* was identified on fig fruits.

To our knowledge, this is the first time that *Fusarium oxysporum* and *Passalora trichopila* have been reported in Romania as important pathogens on fig leaves, completing the informations related to fig tree pathology.

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