Diversity of *Fusarium* Species Isolated from Symptomatic Plants Belonging to a Wide Range of Agri-Food and Ornamental Crops in Lebanon

Charlie Abi Saad 1,2,†, Mario Masiello 1,†, Wassim Habib 3,4, Elvis Gerges 4, Simona Marianna Sanzani 2,5, Antonio Francesco Logrieco 1, Antonio Moretti 1,*, and Stefania Somma 1,*

1 Institute of Science of Food Production-ISPA, Research National Council—CNR, Via Amendola 122/O, 70126 Bari, Italy
2 International Center for Advanced Mediterranean Agronomic Studies-CIHEAM Bari, Via Ceglie 9, Valenzano, 70010 Bari, Italy
3 Centro di Ricerca, Sperimentazione e Formazione in Agricoltura “Basile Caramia”-CRSFA, Via Cisternino 281, Locorotondo, 70010 Bari, Italy
4 Laboratory of Mycology, Department of Plant Protection, Lebanese Agricultural Research Institute, Fanar 1202, Lebanon
5 Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy
* Correspondence: antonio.moretti@ispa.cnr.it (A.M.); stefania.somma@ispa.cnr.it (S.S.)
† These authors contributed equally to this work.

Abstract: Lebanon is a small Mediterranean country with different pedoclimatic conditions that allow the growth of both temperate and tropical plants. Currently, few studies are available on the occurrence and diversity of *Fusarium* species on Lebanese crops. A wide population of *Fusarium* strains was isolated from different symptomatic plants in the last 10 years. In the present investigation, a set of 134 representative strains were molecularly identified by sequencing the translation elongation factor, used in *Fusarium* as a barcoding gene. Great variability was observed, since the strains were grouped into nine different *Fusarium* Species Complexes (SCs). *Fusarium oxysporum* SC and *Fusarium solani* SC were the most frequent (53% and 24%, respectively). Members of important mycotoxigenic SCs were also detected: *F. fujikuroi* SC (7%), *F. sambucinum* SC (5%), *F. incarnatum-equiseti* SC (3%), and *F. tricinctum* SC (4%). Two strains belonging to *F. lateritium* SC, a single strain belonging to *F. burgessii* SC, and a single strain belonging to *F. redolens* SC were also detected. This paper reports, for the first time, the occurrence of several *Fusarium* species on Lebanese host plants. The clear picture of the *Fusarium* species distribution provided in this study can pose a basis for both a better understanding of the potential phytopathological and toxicological risks and planning future *Fusarium* management strategies in Lebanon.

Keywords: Lebanese plants; *Fusarium* species complexes; mycotoxins

1. Introduction

Lebanon is one of the smallest countries in the Mediterranean basin. Although it has a limited agricultural area of about 650,000 ha [1], the variety of agricultural land and climatic conditions enables farmers to grow a plethora of plants, including both temperate crops (e.g., grape, olive tree, and solanaceous plants) and tropical crops (e.g., banana and avocado). The territory is quite varied, ranging from the flat coastal area to the mountain chain in the center of the country, where large areas are occupied by cedars and fir woods. Generally, tobacco and figs are grown in the southern part of Lebanon, citrus fruits and banana along the coast, olives and apples in different parts of the Mount Lebanon governorate, and, in the north, fruits, vegetables, and cereals in the fertile Bekaa Valley. Lebanon exports part of its production to other Middle Eastern countries and Arab Gulf states [1].
Several factors, including pedo-climatic conditions, poor agricultural and storage practices, and the weakness of the food safety management system, can simultaneously lead to contamination of Lebanese agri-food commodities by toxigenic fungi and their mycotoxins [2]. This represents both a phytopathological issue and a serious risk to consumers’ health. In recent decades, the socio-economic disorder due to the long civil war and the political instability have not allowed the improvement of the weak national food safety system. Only in 2015, for the first time, was the Food Safety Lebanese Commission established, although the new political instability is compromising its activities [2].

Under Lebanese climatic conditions, all crops could be potentially colonized by the most important mycotoxigenic fungal species belonging to the *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* genera. However, to date, few studies are available in the literature on the biodiversity of phytopathogenic fungi and the accumulation of mycotoxins in Lebanese crops and agri-food commodities. Recently, thorough surveys and phylogenetic studies were carried out by Habib et al. [3–5] to evaluate the distribution and the toxicological risk of *Alternaria* and *Penicillium* species on wheat and tomato at preharvest, and apple and table grape at postharvest. On the other hand, El Darra et al. [6] evaluated the occurrence of mycotoxins in spices and herbs commercialized in Lebanon, showing the high level of mycotoxins in spices consumed in Lebanon. A few studies have also reported the occurrence of *Aspergillus* and *Penicillium* species and their mycotoxins on wheat, grape, and other commodities [7–11]. On the contrary, very little information is available on the occurrence and diversity of *Fusarium* species on Lebanese crops. Such information includes the report by Ordonez et al. [12] on the occurrence of *Fusarium oxysporum* f.sp. *cubense* on banana, and the reports on *Fusarium redolens* and *F. oxysporum* isolated from chickpea [13,14].

The *Fusarium* genus includes several phytopathogenic species, such as *F. graminearum* and *F. oxysporum*, which are two causal agents of the most important plant diseases worldwide [15]. Nowadays, based on genetic analyses, taxonomists have grouped *Fusarium* species into 23 well-separated phylogenetic species complexes [16,17]. Worldwide, the most important phytopathogenic *Fusarium* species belong to the *Fusarium sambucinum* species complex (FSAMSC), *Fusarium fujikuroi* species complex (FFSC), *Fusarium solani* species complex (FSSC), and *Fusarium oxysporum* species complex (FOSC). To a lesser extent, members of the *Fusarium incarnatum-equiseti* species complex (FIESC), regarded as moderate pathogens, have been isolated together with other *Fusarium* species from different diseased plants, such as cereals, sugarcane, and date palm [18–21].

In particular, *Fusarium* species included in the FSAMSC and FFSC represent both a phytopathological issue and a serious toxicological risk for humans and animals, although these two aspects are rarely related. Indeed, almost all 78 and 56 species included in the FSAMSC and FFSC, respectively, can simultaneously produce multiple mycotoxins [16]. In particular, the most important mycotoxins produced by FSAMSC are type A and type B trichothecenes, such as HT-2 and T-2 toxins, nivalenol, deoxynivalenol (DON) and their acetylated forms, and zearalenone [22]. The most dangerous mycotoxins produced by FFSC are fumonisins B (FBs), occurring worldwide, primarily in maize, and especially in warm climates [23]. The most important among FBs is FB$_1$, classified by IARC as a possible carcinogen to humans [24]. Together with FBs, members of the FFSC are also able to produce so-called emerging mycotoxins, such as beauvericin, enniatins, fusicarid, and moniliformin [25]. These emerging mycotoxins can be synthesized by species belonging to different species complexes, such as the FOSC, FIESC, and *Fusarium tricinctum* species complex (FTSC) [16]. BEA and ENNs B are reported to possess phytotoxic properties, acting as virulence factors in tomato and potato plant infections [26,27].

Members of the FOSC and FSSC are of great concern, because they are the most important causal agents of vascular wilt and root rot in diverse crops. The most important diseases caused by *F. oxysporum* include *Fusarium* wilt of banana, common bean, cotton, lettuce, and tomato. However, it must be highlighted that the FOSC also includes many populations of strains that are not pathogenic, with the pathogenic ones usually belonging to host-specific formae speciales. The FSSC includes causal agents of soybean sudden
death syndrome, bean root rot, and multiple vegetable diseases \cite{28,29}. In addition, some members of the FSSC can be involved in human and animal infections.

Since the Fusarium species distribution is widely influenced by environmental conditions, and several species showing different mycotoxin profiles can be largely isolated together from symptomatic plants, a correct identification of Fusarium species can allow for a clear picture of the potential phytopathological and toxicological risks. From this perspective, the aim of this study was to identify a wide set of Fusarium strains isolated from different agriculturally important crops in Lebanon to obtain, for the first time, a large amount of information on the Fusarium species diversity in this country.

2. Materials and Methods

2.1. Sampling

During a 10-year period, from 2012 to 2021, and within the service of the plant disease diagnostic clinic provided by the Lebanese Agricultural Research Institute (LARI), symptomatic plant tissues were brought by private companies, public institutions, and farmers from all over Lebanon (Figure 1). Fusarium colonies, isolated from different host plants, including agri-food plants, ornamental plants, and forest trees, were selected to obtain a collection of 134 Fusarium strains (Table 1).

![Figure 1](Image of Lebanon map with marked sites of host plant sampling.)

Table 1. List of Fusarium strains isolated from Lebanese plants. The geographical origin, host plant, observed symptoms, part of the plant from which the strains were isolated, and the identified Fusarium species complex are reported for each Fusarium strain.

| Strain Code | Lebanese Region | Host Plant | Organ | Symptoms | Fusarium Species Complex |
|------------|-----------------|------------|-------|----------|--------------------------|
| CA135      | Bekaa           | Eggplant   | Crown area | Wilt, decline, death | FOSC                     |

2. Materials and Methods

2.1. Sampling

During a 10-year period, from 2012 to 2021, and within the service of the plant disease diagnostic clinic provided by the Lebanese Agricultural Research Institute (LARI), symptomatic plant tissues were brought by private companies, public institutions, and farmers from all over Lebanon (Figure 1). Fusarium colonies, isolated from different host plants, including agri-food plants, ornamental plants, and forest trees, were selected to obtain a collection of 134 Fusarium strains (Table 1).
| Strain Code | Lebanese Region | Host Plant | Organ | Symptoms | Fusarium Species Complex |
|-------------|-----------------|------------|-------|----------|--------------------------|
| CA135       | Bekaa           | Eggplant   | Crown area | Wilt, decline, death | FOSC                     |
| CA136       | Bekaa           | Eggplant   | Crown area | Wilt, decline, death | FOSC                     |
| CA53        | Akkar           | Eggplant   | Roots, crown | Vascular browning, wilt, decline | FOSC                     |
| CA7         | Bekaa           | Eggplant   | Stem, petioles | Wilt, decline | FSAMSC                   |
| CA42        | South           | Potato     | Roots, crown | Internal browning | FOSC                     |
| CA13        | Akkar           | Potato     | Seed tubers | Dry rot | FSSC                     |
| CA25        | Akkar           | Potato     | Roots, crown | Wilt, decline | FOSC                     |
| CA26        | Akkar           | Potato     | Roots, crown | Wilt, decline | FOSC                     |
| CA27        | Akkar           | Potato     | Roots, crown | Wilt, decline | FOSC                     |
| CA28        | Akkar           | Potato     | Roots, crown | Wilt, decline | FOSC                     |
| CA29        | Akkar           | Potato     | Roots, crown | Wilt, decline | FOSC                     |
| CA30        | Bekaa           | Potato     | Roots, crown | Wilt, death | FOSC                     |
| CA41        | South           | Potato     | Roots, crown | Internal browning | FOSC                     |
| CA120       | Bekaa           | Potato     | Stem | Necrotic leaves, necrotic spots on stem | FOSC                     |
| CA9         | Akkar           | Potato     | Roots, tubers | Tuber seeds unusual growth | FSAMSC                   |
| CA130       | Bekaa           | Potato     | Tubers | Spoiled tubers, white mycelium on tubers | FSAMSC                   |
| CA37        | South           | Potato     | Roots, crown | Internal browning | FSSC                     |
| CA38        | South           | Potato     | Roots, crown | Internal browning | FSSC                     |
| CA39        | South           | Potato     | Roots, crown | Internal browning | FSSC                     |
| CA40        | South           | Potato     | Roots, crown | Internal browning | FSSC                     |
| CA22        | Akkar           | Potato     | Seed tubers | Dry rot | FSSC                     |
| CA114       | Bekaa           | Potato     | Roots, crown | Decline, black lesions on roots | FOSC                     |
| CA10        | Akkar           | Potato     | Roots, tubers | Tuber seeds unusual growth | FSAMSC                   |
| CA102       | Akkar           | Potato     | Tuber | Necrotic spots and lesions | FSAMSC                   |
| CA55        | Akkar           | Tobacco    | Collar area | Wilt, yellowing, chlorotic leaves, death | FSSC                     |
| CA61        | Mount Leb.      | Tomato     | Collar area | Poor growth, weakness | FOSC                     |
| CA134       | North           | Tomato     | Roots | Wilt, decline, death | FOSC                     |
| CA67        | Mount Leb.      | Tomato     | Roots, crown | Wilt, internal browning | FOSC                     |
| CA11        | Bekaa           | Tomato     | Roots, stem | Leaf chlorosis, necrosis, wilt | FOSC                     |
| CA14        | Mount Leb.      | Tomato     | Roots, stem | General decline, wilt | FOSC                     |
| CA24        | Mount Leb.      | Tomato     | Roots, stem | Necrosis on crown area, wilt | FOSC                     |
| CA47        | North           | Tomato     | Stem | Vascular browning, wilt, decline | FOSC                     |
| CA88        | Bekaa           | Tomato     | Roots | Corky roots | FSSC                     |
| CA54        | Akkar           | Hot pepper | Roots, crown | Vascular browning, wilt, decline | FOSC                     |
| CA32        | South           | Banana     | Fruits | Fruit tip and crown necrosis | FFSC                     |
| CA132       | South           | Banana     | Pseudo stem | Decline, yellowing | FFSC                     |
| Strain Code | Lebanese Region | Host Plant | Organ | Symptoms | Fusarium Species Complex |
|-------------|----------------|------------|-------|----------|-------------------------|
| CA105       | South          | Banana     | Crown area | Yellowing, decline, death | FOSC                    |
| CA106       | South          | Banana     | Crown area | Yellowing, decline, death | FOSC                    |
| CA46        | South          | Banana     | Crown, stem | Wilting, yellowing, Leaf yellowing, pseudo stem cracking, internal browning | FOSC                    |
| CA127       | South          | Banana     | Pseudo stem | Leaf yellowing, pseudo stem cracking, internal browning | FOSC                    |
| CA128       | South          | Banana     | Pseudo stem | Leaf yellowing, pseudo stem cracking, internal browning | FOSC                    |
| CA129       | South          | Banana     | Pseudo stem | Leaf yellowing, pseudo stem cracking, internal browning | FOSC                    |
| CA31        | South          | Banana     | Roots, crown | Wilt, internal browning | FOSC                    |
| CA119       | South          | Banana     | Stem | Internal discoloration, decline, death | FOSC                    |
| CA98        | South          | Banana     | Stem | Yellowing, decline, death | FFSC                    |
| CA99        | South          | Banana     | Stem | Yellowing, decline, death | FFSC                    |
| CA100       | South          | Banana     | Crown area | Yellowing, decline, death | FOSC                    |
| CA103       | South          | Banana     | Crown area | Yellowing, decline, death | FOSC                    |
| CA97        | South          | Banana     | Stem | Yellowing, decline, death | FOSC                    |
| CA92        | South          | Banana     | Crown area | Internal browning and necrosis | FSSC                    |
| CA93        | South          | Banana     | Crown area | Internal browning and necrosis | FSSC                    |
| CA95        | South          | Banana     | Crown area | Yellowing, decline, death | FSSC                    |
| CA96        | South          | Banana     | Crown area | Yellowing, decline, death | FSSC                    |
| CA60        | Bekaa          | Lettuce    | Collar area | Wilt, decline, death | FFSC                    |
| CA52        | Bekaa          | Lettuce    | Collar area | Dry rot, wilt, chlorotic leaves | FOSC                    |
| CA57        | Bekaa          | Lettuce    | Collar area | Yellowing, wilting, dry rot | FOSC                    |
| CA74        | Bekaa          | Lettuce    | Crown area | Yellowing, wilting, dry rot | FOSC                    |
| CA77        | Bekaa          | Lettuce    | Crown area | Yellowing, stunting, death | FOSC                    |
| CA85        | Bekaa          | Lettuce    | Crown area | Wilt, decline, death | FOSC                    |
| CA108       | Bekaa          | Lettuce    | Crown area | Dry rot | FOSC                    |
| CA115       | Bekaa          | Lettuce    | Crown area | Wilt, decline, death | FOSC                    |
| CA116       | Bekaa          | Lettuce    | Crown area | Wilt, decline, death | FOSC                    |
| CA125       | Bekaa          | Lettuce    | Crown area | Decline, death, vascular discoloration | FOSC                    |
| CA33        | Bekaa          | Lettuce    | Roots, crown | Root rot | FOSC                    |
| CA82        | Bekaa          | Lettuce    | Roots, crown | Wilt, decline, death | FOSC                    |
| CA107       | Bekaa          | Lettuce    | Roots, crown | Decline, internal browning | FOSC                    |
| CA56        | Bekaa          | Lettuce    | Collar area | Yellowing, wilting, dry rot | FSSC                    |
| Strain Code | Lebanese Region | Host Plant | Organ       | Symptoms                                      | Fusarium Species Complex |
|-------------|-----------------|------------|-------------|-----------------------------------------------|--------------------------|
| CA58        | Bekaa           | Lettuce    | Collar area | yellowing, wilting, dry rot                    | FSSC                     |
| CA79        | South           | Lettuce    | Crown area  | Wilt, decline, death                          | FSSC                     |
| CA118       | Bekaa           | Lettuce    | Crown area  | Black necrotic spots on collar area           | FSSC                     |
| CA65        | Bekaa           | Fir        | Roots       | Collapse, death                              | FOSC                     |
| CA36        | Mount Leb.      | Hornbeam   | Roots, crown| Wilt, dieback, necrotic leaves                | FIESC                    |
| CA66        | Akkar           | Cedars     | Roots       | Collapse, sudden death                        | FFSC                     |
| CA71        | Bekaa           | Cedars     | Roots, crown| Yellowing, decline, death                     | FSSC                     |
| CA68        | Bekaa           | Cedars     | Roots, crown| Yellowing, decline, death                     | FSSC                     |
| CA69        | Bekaa           | Cedars     | Roots, crown| Yellowing, decline, death                     | FSSC                     |
| CA70        | Bekaa           | Cedars     | Roots, crown| Yellowing, decline, death                     | FSSC                     |
| CA72        | Bekaa           | Pine       | Crown area  | Yellowing, decline, death                     | FSSC                     |
| CA73        | Bekaa           | Pine       | Crown area  | Yellowing, decline, death                     | FSSC                     |
| CA86        | Mount Leb.      | Pine seedlings | Stem, roots | Wilt, death, corky roots                      | FOSC                     |
| CA111       | Bekaa           | Walnut     | Crown area  | Crown rot                                     | FSSC                     |
| CA8         | Bekaa           | Cucumber   | Roots       | Wilting                                       | FSSC                     |
| CA117       | Mount Leb.      | Cucumber   | Crown area  | Soft rot                                      | FOSC                     |
| CA121       | South           | Melon      | Fruits      | Black spots, circular blackish lesions        | FSAMSC                   |
| CA122       | South           | Melon      | Fruits      | Black spots, circular blackish lesions        | FIESC                    |
| CA35        | Bekaa           | Melon      | Roots       | Dry rot, browning                            | FOSC                     |
| CA34        | Bekaa           | Melon      | Roots       | Dry rot, browning                            | FSSC                     |
| CA50        | South           | Watermelon | Roots, crown| Wilted and stunted plants, root rot           | FOSC                     |
| CA51        | South           | Watermelon | Roots, crown| Wilted and stunted plants, root rot           | FOSC                     |
| CA23        | South           | Watermelon | Roots, stem | Wilt, dry rot                                | FOSC                     |
| CA19        | Akkar           | Citrus     | Seedling roots | No symptoms                                | FIESC                    |
| CA16        | South           | Citrus     | Seedling roots | No symptoms                                | FOSC                     |
| CA6         | South           | Grapefruit | Fruits      | Fruit spots, lesions                         | FTSC                     |
| CA4         | South           | Lemon      | Fruits      | Fruit spots, lesions                         | FLSC                     |
| CA3         | South           | Lemon      | Stem, twigs | Wilt, dieback, decline                       | FLSC                     |
| CA1         | Akkar           | Lemon      | Stem, twigs | Wilt, dieback, decline                       | FTSC                     |
| CA2         | Akkar           | Lemon      | Stem, twigs | Wilt, dieback, decline                       | FTSC                     |
| CA5         | South           | Mandarin   | Branch      | Gummosis, exudates                           | FTSC                     |
| CA59        | South           | Beans      | Collar area | Wilt, rot                                     | FOSC                     |
| CA84        | Mount Leb.      | White beans | Roots, crown| Wilt, decline, death                          | FOSC                     |
| CA44        | South           | Faba beans | Roots       | Root rot                                      | FOCS                     |
| CA45        | North           | Peas       | Roots, crown| General wilt                                 | FOSC                     |
| CA94        | Mount Leb.      | Peas       | Roots       | Slow growth                                  | FOSC                     |
| CA126       | Mount Leb.      | Peas       | Roots, collar area | Chlorotic dark region on the lower part of the stem | FSSC                     |
| CA64        | South           | Strawberry | Crown area  | Dry rot, wilt, death                         | FFSC                     |
| CA63        | Mount Leb.      | Strawberry | Crown area  | Wilt, death                                   | FOSC                     |
Table 1. Cont.

| Strain Code | Lebanese Region | Host Plant       | Organ                  | Symptoms                             | Fusarium Species Complex |
|-------------|-----------------|------------------|------------------------|--------------------------------------|--------------------------|
| CA76        | Bekaa           | Strawberry       | Crown area             | Wilt, decline, death                 | FOSC                     |
| CA123       | South           | Strawberry       | Crown area             | Wilt, death, dry rot in vascular tissue | FOSC                     |
| CA109       | North           | Strawberry       | Roots, crown           | Decline, death                       | FOSC                     |
| CA112       | Mount Leb.      | Ivy              | Roots, crown           | Decline, death, roots and stem cracking | FSSC                    |
| CA89        | Beirut          | Jacaranda tree   | Roots                  | Wilt, decline, death                 | FSSC                     |
| CA12        | Mount Leb.      | Poinsettia       | Roots                  | Dieback                              | FOSC                     |
| CA75        | Mount Leb.      | Poinsettia       | Crown area             | Wilt, decline, vascular browning     | FSSC                     |
| CA104       | Mount Leb.      | Roses            | Crown area             | Decline, death                       | FSAMSC                   |
| CA91        | Bekaa           | Onion            | Bulb                   | Internal brown rot                   | FOSC                     |
| CA43        | Bekaa           | Onion            | Bulbs                  | Rot                                  | FOSC                     |
| CA90        | Bekaa           | Onion            | Bulb                   | Internal brown rot                   | FSSC                     |
| CA83        | Bekaa           | Onion            | Roots, crown           | Yellowing, weak root system          | FSSC                     |
| CA17        | South           | Olive            | Seedling roots         | No symptom                           | FFSC                     |
| CA15        | South           | Olive            | Seedling roots         | No symptom                           | FOSC                     |
| CA18        | South           | Olive            | Seedling roots         | No symptom                           | FOSC                     |
| CA21        | South           | Olive            | Seedling roots         | No symptom                           | FOSC                     |
| CA110       | Mount Leb.      | Apple            | Roots                  | Wilt, death                          | FSSC                     |
| CA48        | Mount Leb.      | Red apple        | Fruits                 | Fruit rot                            | FTSC                     |
| CA49        | Mount Leb.      | Red apple        | Fruits                 | Fruit rot                            | FTSC                     |
| CA133       | Mount Leb.      | Avocado          | Roots                  | Symptoms associated with ambrosia    | FSSC                     |
| CA87        | South           | Avocado          | Stem bark              | Twigs and stem boring                | FSSC                     |
| CA131       | Mount Leb.      | Basil            | Stem, crown area       | Black necrotic lesions on stem       | FOSC                     |
| CA62        | Bekaa           | Cauliflower      | Roots, crown           | Wilt, internal browning              | FOSC                     |
| CA113       | Mount Leb.      | Kiwi             | Twigs                  | Decline, dieback                     | FOSC                     |
| CA124       | Mount Leb.      | Parsley          | Roots                  | Yellowing                            | FOSC                     |
| CA78        | Mount Leb.      | Parsley          | Roots, crown           | Wilt, decline, death                 | FRSC                     |
| CA81        | Mount Leb.      | *Paspalum* grass | Roots                  | Leaf yellowing, root rot             | FIESC                    |
| CA80        | Mount Leb.      | *Paspalum* grass | Roots                  | Leaf yellowing, root rot             | FOSC                     |

2.2. Morphological Identification of Fusarium Strains

For fungal isolation, after surface disinfection with 70% ethanol or 2% sodium hypochlorite solution and washing twice with sterile distilled water, small portions (about 5 mm × 5 mm) taken from the margin of the infected areas were transferred to potato dextrose agar plates (PDA, Merck, Darmstadt, Germany) and incubated for 5–7 days at 25 °C until colony development.

To obtain pure Fusarium strains from the developing fungal colonies, a mass of conidia was resuspended in sterile distilled water and scattered at low density on 90 mm water agar (WA) Petri dishes. After 18–24 h of incubation at 25 °C in the darkness, germinated conidia were singularly transferred to 60 mm PDA plates using a dissecting microscope.

After 5–7 days of incubation at 25 °C under a 12 h photoperiod, Fusarium strains cultured on PDA and Spezieller Nahrstoffarmer agar (SNA) media were morphologically identified according to Nelson et al. and Leslie and Summerell [30,31].
2.3. Molecular Identification of the Fusarium Species by Translation Elongation Factor 1-α Sequencing

2.3.1. DNA Extraction

For each monosporic strain, 5 mycelium plugs were collected from the margin of actively growing colonies on PDA and transferred to a sterilized cellophane disk overlaid on PDA 90 mm plates. After incubation at 25 °C for 2–3 days, the mycelia were collected into 2 mL tubes, frozen, and lyophilized. The DNA extraction was carried out on 15 mg of powdered lyophilized mycelium by using a Wizard Magnetic DNA Purification System for Food kit (Promega Corporation, Madison, WI, USA) according to the manufacturer’s protocol. The genomic DNA quantity and integrity were checked on 0.8% agarose gel in 1X Tris-Acetate-EDTA (TAE) using a standard 1 kb DNA Ladder (ThermoFisher Scientific, Carlsbad, CA, USA) and a Nanodrop spectrophotometer (ThermoFisher Scientific).

2.3.2. Translation Elongation Factor 1-α (TEF) Amplification and Sequencing Analysis

For each Fusarium strain, the informative fragment of the Translation elongation factor 1-α gene was amplified and sequenced using the primer pair EF-1 and EF-2 [32]. PCR reactions were carried out in a 15 µL final volume containing 15 ng of genomic DNA, 300 nM of each primer, 200 nM dNTPs, 1 × PCR buffer, and 0.6 U of Hot Start Taq DNA Polymerase (Fisher Molecular Biology, Trevose, PA, USA). The amplifications were carried out in a Mastercycler EP Gradient thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation of 2 min at 95 °C, followed by 35 cycles of 50 s at 95 °C, 50 s at 59 °C, 1 min at 72 °C, and a final extension of 7 min at 72 °C.

The PCR products, stained with GelRed® (Biotium Inc., Fremont, CA, USA), were checked after electrophoretic separation on 1.5% agarose gel in 1 × TAE buffer under UV light by comparison with a 100 bp DNA Ladder (ThermoFisher Scientific). Before sequencing, the PCR products were purified with an enzymatic EXO/FastAP mixture (ExonucleaseI and FastAP thermosensitive alkaline phosphatase, ThermoFisher Scientific). Sequence reactions were performed for both strands using a BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s recommendations. The labeled products were purified by filtration through Sephadex G-50 (5%) (Sigma-Aldrich, Saint Louis, MO, USA) and analyzed using an ABI PRISM 3730 Genetic Analyzer (ThermoFisher Scientific). The FASTA sequences obtained were analyzed and assembled using BioNumerics v. 5.1 software (Applied Maths, Kortrijk, Belgium).

For molecular identification at the species level, the obtained sequences were compared with the sequences available in the NCBI Database through the BLASTN program. To further solve the phylogenetic relationships between and within the Fusarium species detected, the sequences obtained in this study joined to 23 sequences of Fusarium reference strains and to the sequence of Ilyonectria crassa CBS 158.31 strain, used as an outgroup, were aligned by using the MUSCLE algorithm [33]. Phylogenetic relationships were analyzed using the maximum likelihood method with MEGA software version 7 [34]. Bootstrap analyses [35] were conducted to determine the confidence of internal nodes using a heuristic search with 1000 replicates, removing gaps.

3. Results

In Table 1, 134 Fusarium strains, isolated from 38 different host plants, are listed. In particular, the strains were isolated from different plant parts, such as the roots, crown area, stem, and fruits, showing symptoms of fungal diseases, such as wilting, vascular browning, yellowing, necrotic or chlorotic leaf spots, crown rot, and also decline or death. For each Fusarium strain, the geographical and host plant origin, organ of isolation, and symptoms observed are detailed. The majority of the strains were isolated from plants grown in the South and Bekaa regions (45 and 42 strains, respectively), followed by Mount Lebanon (25 strains) and Akkar (17 strains) regions. Four Fusarium strains were also collected in...
the North, and a single strain was obtained from an ornamental plant grown in Beirut city (Table 1).

Host plants affected by Fusarium species were grouped considering their botanical and commodity classifications, as shown in Figure 2. Most of the Fusarium strains were isolated from solanaceous plants (34 out of 134), banana (19 out of 134) and lettuce (17 out of 134), together representing half of the Fusarium strains isolated from symptomatic plants. In detail, 20 strains were isolated from potato, 8 strains from tomato, 4 strains from eggplant, and a single Fusarium strain from tobacco and hot pepper, respectively (Table 1). To a lesser extent, Fusarium strains were isolated from forest tree (11 strains), cucurbits (9 strains), citrus trees (8 strains), legume (6 strains), strawberry (5 strains), ornamental plants (5 strains), and onion and olive tree (4 strains, respectively). As reported in Table 1, 12 Fusarium strains were also isolated from other host plants, including apple (3 strains), avocado, parsley, and Paspalum grass (2 strains from each), and basil, cauliflower, and kiwi (a single strain from each). The distributions of Fusarium species in the different geographical regions are reported in Table 2. The single strain isolated in Beirut, from the ornamental Jacaranda tree, was identified as a member of the FFSC. In the North Lebanon region, four Fusarium strains were isolated from tomato, pea, and strawberry, and identified as FOSC. A large number of Fusarium strains were isolated from plants grown in the Akkar, Bekaa, Mount Lebanon, and South Lebanon regions. In all of these regions, the FOSC and FSSC were the most dominant SCs, with values ranging between 43.8 (Akkar) and 59.5% (Bekaa), and between 12.5 (Akkar) and 31% (Bekaa) (Table 2). In addition to the FOSC and FSSC strains, in the Southern region, great variability among Fusarium species isolated was observed, with high frequency of the FFSC (13.3%), followed by the FTSC and FLSC (4.4%), and, to a lesser extent, by FIESC, FSAMSC, and FBSC (2.2%). Fusarium redolens strains were isolated only from plants grown in the Mount Lebanon region, with a value of 4%. In this region, strains belonging to the FTSC (8%), FIESC (8%), and FSAMSC (4%) were also isolated. In the Bekaa region, strains belonging to the FSAMSC and FFSC, with values of 4.8%, were isolated. In the Akkar region, 6.3% of the strains belonged to the FIESC, and the same value of 12.5% of strains was recorded for each of the following SCs: FSAMSC, FFSC, and FTSC (Table 2).

Figure 2. Lebanese host plants from which Fusarium strains (shown for each group of plants in the graph) were isolated: solanaceous plants (potato 20, tomato 8, eggplant 4, tobacco 1, and hot pepper 1); banana, lettuce, citrus trees (citrus 2, lemon 4, grapefruit 1, and mandarin 1), legumes (bean 2, pea 3, and faba bean 1), cucurbits (melon 4, watermelon 3, and cucumber 2), strawberry, forest trees (cedar 5, abies 1, hornbeam 1, pine 3, and walnut tree 1), ornamental plants (ivy 1, poinsettia 2, Jacaranda 1, and roses 1), olive tree, and onion. To a lesser extent, Fusarium strains were isolated from other host plants (apple 3, avocado 3, kiwi 1, cauliflower 1, basil 1, parsley 1, and paspalum grass 1).
Table 2. Fusarium species distribution in the different Lebanese regions.

| Geographical Origin | Fusarium Strains | Frequency of Fusarium Species (%) |
|---------------------|------------------|----------------------------------|
|                     | Strains          | FOSC    | FSSC    | FSAMSC  | FFSC    | FTSC    | FIESC   | FLSC    | FRSC    | FBSC    |
| South               | 45               | 48.9    | 22.2    | 2.2     | 13.3    | 4.4     | 2.2     | 4.4     | 0       | 2.2     |
| Bekaa               | 42               | 59.5    | 31      | 4.8     | 4.8     | 0       | 0       | 0       | 0       | 0       |
| Mount Lebanon       | 25               | 52      | 24      | 4       | 0       | 8.0     | 8.0     | 0       | 4       | 0       |
| Akkar               | 17               | 43.7    | 12.5    | 12.5    | 12.5    | 6.3     | 0       | 0       | 0       | 0       |
| North               | 4                | 100     | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 0       |
| Beirut              | 1                | 0       | 100     | 0       | 0       | 0       | 0       | 0       | 0       | 0       |

The phylogenetic analysis of the TEF sequences of 134 Lebanese Fusarium strains, 23 Fusarium reference sequences, and 1 sequence of Ilyonectria crassa used as an outgroup taxon resulted in the tree shown in Figure 3. The phylogenetic tree was resolved in 11 well-separated clades, supported by high bootstrap values (over 98%). A large set of 71 strains clustered together and with the reference strain NRRL 52787, belonging to the F. oxysporum species complex (FOSC). Ten strains were identified as belonging to the F. fujikuroi species complex (FFSC); in particular, two strains were identified as F. steriliphosum, one strain as F. musae, two as F. sacchari, and five strains showed high similarity to the F. proliferatum reference strain. Another 20 strains clustered in six well-supported clades, corresponding to the F. burgessii species complex (FBSC), F. redolens species complex (FRSC), F. tricinctum species complex (FTSC), F. incarnatum-equiseti species complex (FIESC), F. sambucinum species complex (FSAMSC), and F. lateritium species complex (FLSC). The FBSC and FRSC were both represented by only one strain and the corresponding species reference strain (F. algeriense and F. redolens, respectively). In detail, six strains belonged to the FTSC, among which two strains showed homology with the F. acuminatum NRRL 36147 reference strain. Among four FIESC strains, one strain showed high similarity to the F. citri reference strain, one strain to F. pernambucanum, and one strain to F. clavum. The FSAMSC clade included one strain identified as F. culmorum, two strains clustered with the F. sambucinum reference strain, and three strains sharing high homology with the reference strain F. tumidum NRRL 38939. One strain showed similarity to the reference strains NRRL 66923 and NRRL 46662, belonging to the FSAMSC, but this well-supported clade (100 bootstrap value) did not group with the other species of the FSAMSC described previously. Two strains were identified as belonging to the FLSC clade. Another large clade grouping 32 Lebanese strains was the Fusarium solani species complex (FSSC). Most of these strains showed similarity to the F. suttonianum reference strain NRRL 32858; two strains were identified as F. euwallaceae, and three strains clustered with F. solani NRRL 45880.

The occurrence of the different Fusarium species complexes identified through the phylogenetic analysis of TEF sequences in Lebanese plants is shown in Figure 4. The FOSC was the most widespread species complex in Lebanon, representing 53% of the overall 134 collected strains, followed by the FSSC, with a percentage of 24%. Other species complexes occurring in different plants at 7, 5, and 4% are the FFSC, FSAMSC, and FTSC, respectively. To a lesser extent, strains belonging to the FIESC (3%) and FLSC (2%) were identified. Finally, one strain of F. redolens from parsley crown and one strain of F. algeriense from potato crown and roots were isolated.
Figure 3. Phylogenetic tree built with the TEF sequences of 134 Lebanese *Fusarium* strains and 23 species reference strains. The phylogenetic tree was generated by the maximum likelihood method; bootstrap values > 70 with 1000 replicates are shown near the branches. *Fusarium* species complexes are also shown in the clustering.

Figure 4. Occurrence of *Fusarium* species complexes, molecularly identified by TEF sequences, in Lebanese plants.
4. Discussion

A wide population of *Fusarium* strains infecting plants all over Lebanon in the last 10 years has been isolated and identified to achieve, for the first time, extended information regarding *Fusarium* diversity occurring on Lebanese crops. Furthermore, since coniferous forests are a primary constituent of the Lebanese landscape, the cedar of Lebanon is included in this study, being the national emblem of the country. Indeed, monitoring activities to assess *Fusarium*’s occurrence in a wide range of plants in Lebanon were lacking, as well as updated information regarding the risk related to *Fusarium* mycotoxins. Plants showing mostly symptoms of decline and withering, were collected from different areas of the country and belonged to a wide range of categories, ranging from agri-food to ornamental plants, including herbaceous plants and forest trees. The most represented commodities in the *Fusarium* host collection were potato, banana, and lettuce. In particular, potato is one of the primary crops grown in Lebanon for local consumption, processing, and export, representing more than 45% of the total area assigned to vegetable crops [1], and banana is one of the main crops intended for both local consumption and export, mostly in South Lebanon. Therefore, it is highly worrisome that such important crops for Lebanese agriculture showed such high *Fusarium* occurrence.

The molecular identification, based on TEF sequencing, allowed distinguishing several *Fusarium* species that, according to the contemporary *Fusarium* species concept [16], have been assigned to given *Fusarium* species complexes. A total of nine different species complexes occurred among the *Fusarium* strains herein, demonstrating that a wide biodiversity of *Fusarium* is detectable in Lebanon. The most represented species complex (53%) was the FOSC, whose members were isolated from almost all of the plants considered in this study. Members of this complex are well-known causal agents of a wide range of diseases, including Panama disease on banana, and Fusarium wilt and root and crown rot on legumes, lettuce, solanaceous plants, and cucurbits [36]. In our study, the plants from which *F. oxysporum* strains were recovered are commonly known in the literature as host crops of this ubiquitous species. Furthermore, these strains were isolated from the crowns and roots of plants showing yellowing, decline, wilt, and rot, which are symptoms of diseases caused by *F. oxysporum* (Table 1). Besides the economic damage caused by their pathogenicity to plants, FOSC members are able to produce toxic metabolites, such as beauvericin (BEA), a phytotoxic compound contributing to pathogenicity in tomato; fusaric acid (FA), known for its ability to induce wilt symptoms in several plants; and enniatins (ENNs). In addition, each of these metabolites has been proven to be toxic to human and animal cell lines or animals fed with contaminated feed [23]. These results encourage the consideration of prevention measures to be adopted by studying these pathogens and control methods aimed to prevent heavy deterioration and yield losses in Lebanese fields.

The second-most-occurring species complex in Lebanon in the present study was the FSSC (24%). Similar to FOSC members, members of this complex are known to include species causing root and crown rot. Consistently, Lebanese FSSC strains were isolated from several different host plants showing browning, necrosis, decline, and rot. Although the FSSC does not contribute to mycotoxin contamination, this complex includes more than 80 species sharing very high genetic variability and is considered among the most relevant pathogenic group of species in the genus.

Both the FOSC and FSSC are present worldwide, but *F. oxysporum* has been previously reported in Lebanon only associated with Panama disease in banana [12] and in chickpea [13]. In the present investigation, nine strains belonging to FFSC were isolated from banana, potato, lettuce, strawberry, olive tree, and the cedar of Lebanon. Among the four strains isolated from banana tree, the only one from fruits was identified as *F. musae*, confirming the strong association of this species with banana fruits, whereas the other strains isolated from banana stems were *F. sacchari*, *F. sterilinosum*, and *F. proliferatum*. Only one FFSC strain from potato tubers was identified as *F. proliferatum*, similar to the other strains from strawberry, olive tree, and cedar. Although the number of occurring
strains is limited, the presence of *F. proliferatum* is of great concern for its ability to produce different mycotoxins, mainly FBs, which have been associated with a wide number of animal diseases and are classified by IARC as potentially carcinogenic [37].

Two other species complexes identified in Lebanese strains of great importance for the presence of toxigenic species are the FSAMSC and FIESC. However, except for one *F. culmorum* strain from eggplant, which produces DON, the species identified within the SAMSC were mainly *F. sambucinum* and *F. tumidum*, both producing trichotheccene type A mycotoxins. Conversely, FIESC species, detected in citrus trees, melon fruits, and ornamental plants are known to produce multiple mycotoxins, including type A and B trichotheccenes, zearalenone, BEA, and ENNs.

On the other hand, 4% of the studied *Fusarium* strains belonged to the FTSC, characterized by widespread species, but producing only BEA and ENNs. These strains were recovered only from citrus trees and from apple fruits. Similarly, *F. avenaceum* was reported as a postharvest pathogen of apples in Italy [38] and the Netherlands [39].

Furthermore, sporadic strains belonging to the FLSC (two strains from lemon), FRSC (a single strain from parsley), and FBSC (a single *F. algeriense* strain from potato root and crown) were identified. These *Fusarium* species can produce mycotoxins, such as BEA, ENNs, FA, and MON. *Fusarium algeriense*, is a recent species identified only on durum wheat in Algeria, as far as we are aware [40]. On the contrary, *F. redolens*, causing wilt decline and death in the parsley plants investigated in our study, has been previously reported in Lebanon on chickpea [14].

5. Conclusions

Lebanon produces several important agricultural commodities, due to the climatic conditions favorable to agriculture, that are even exported to several other countries. Since investigations into the occurrence of *Fusarium* in Lebanon are limited, an extended investigation of *Fusarium* occurrence was conducted, focused on a widespread monitoring of several Lebanese plants of agri-food and ornamental interest. The *Fusarium* genus was proven to be widely spread, with very high biodiversity, including several species that could represent a risk to consumers due to their potential mycotoxin production. The collected strains were distributed in nine different species complexes, with the FOSC and FSSC being the most present. Other important species complexes of great concern, such as the FFSC, FSAMSC, FIESC, and FTSC, were also identified. To a lesser extent, a few strains belonging to the FLSC, FBSC, and FRSC were detected. Most of the species detected in the present investigation are reported for the first time in Lebanon, since few investigations focused on the *Fusarium* genus in this country. The wide distribution and diversity of *Fusarium* around the country suggest a lack of effective disease management, correct identification, and good agricultural practices to prevent any new occurrence and dispersal of pathogens between fields. In addition, this investigation shows that a policy to evaluate the regulation of *Fusarium* mycotoxins in Lebanon is needed and must be undertaken.

**Author Contributions:** Conceptualization, A.M., S.M.S. and S.S.; methodology, A.M., M.M. and S.S.; validation, M.M. and S.S.; formal analysis, C.A.S., E.G., M.M., W.H. and S.S.; investigation, C.A.S., M.M., W.H., E.G. and S.S.; data curation, C.A.S., E.G., M.M., W.H. and S.S.; writing—original draft, C.A.S., M.M. and S.S.; writing—review and editing, M.M., A.M. and S.S.; visualization, A.M., M.M. and S.S.; supervision, A.M., S.M.S., A.F.L. and S.S., project administration, A.F.L. and A.M.; funding acquisition, A.F.L. and A.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available within the article.
Acknowledgments: These research activities were conducted in the frame of the CHEMEAM Bari MSc program “Innovative Approaches for IPM of Mediterranean Fruit Crops”. The authors would like to thank Carine Saab (Lebanese Agricultural Research Institute) for her collaboration in isolating the *Fusarium* strains and maintaining the fungal collection of the institute.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Food and Agriculture Organization Statistics Division (FAOSTAT). Available online: www.fao.org/faostat (accessed on 9 July 2022).

2. Khoury, A.E.; Daour, R.; Atoui, A.; Hoteit, M. Mycotoxin Database-Lebanon.osf.io/s6rx9. Mycotoxins in Lebanese Food Basket. 2022. Available online: luwebx.ul.edu.lb/sites/default/files/2022-03/20220308-LU-RePa.pdf (accessed on 9 July 2022). [CrossRef]  

3. Habib, W.; Masiello, M.; El Ghorayeb, R.; Gerges, E.; Susca, A.; Meca, G.; Quiles, J.M.; Logrieco, A.F.; Moretti, A.; et al. Occurrence and Characterization of *Penicillium* Species Isolated from Post-Harvest Apples in Lebanon. *Toxins* 2021, 13, 730. [CrossRef] [PubMed]  

4. Habib, W.; Masiello, M.; El Ghorayeb, R.; Gerges, E.; Susca, A.; Meca, G.; Quiles, J.M.; Logrieco, A.F.; Moretti, A. Mycotoxin Profile and Phylogeny of Pathogenic *Alternaria* Species Isolated from Symptomatic Tomato Plants in Lebanon. *Toxins* 2021, 13, 513. [CrossRef] [PubMed]  

5. Habib, W.; Khalil, J.; Mincuzzi, A.; Saab, C.; Gerges, E.; Tsouvalakis, H.C.; Ippolito, A.; Sanzani, S.M. Fungal pathogens associated with harvested table grapes in Lebanon, and characterization of the mycotoxigenic genera. *Phytopathol. Mediterr.* 2021, 60, 427–439. [CrossRef]  

6. Darra, N.E.; Gambacorta, L.; Solfrizzo, M. Multimycotoxins occurrence in spices and herbs commercialized in Lebanon. *Food Control* 2019, 95, 63–70. [CrossRef]  

7. Joubrane, K.; Mnayer, D.; El Khoury, A.; El Khoury, A.; Awad, E. Co-Occurrence of Aflatoxin B1 and Ochratoxin A in Lebanese Stored Wheat. *J. Food Prot.* 2020, 83, 1547–1552. [CrossRef]  

8. Makhlouf, J.; Carvajal-Campos, A.; Querin, A.; Tadrist, S.; Puel, O.; Lorber, S.; Oswald, I.; Hamze, M.; Bailly, J.D.; Bailly, S. Morphologic, molecular and metabolic characterization of *Aspergillus* section *Flavi* in spices marketed in Lebanon. *Sci. Rep.* 2019, 9, 5263. [CrossRef]  

9. Joubrane, K.; Khoury, A.E.; Lteif, R.; Rizk, T.; Kallassy, M.; Hilan, C.; Maroun, R. Occurrence of aflatoxin B1 and ochratoxin A in Lebanese cultivated wheat. *Mycotoxin Res.* 2011, 27, 249–257. [CrossRef]  

10. Khoury, A.E.; Rizk, T.; Lteif, R.; Azouri, H.; Delia, M.L.; Lebrihi, A. Occurrence of ochratoxin A- and aflatoxin B1-producing fungi in Lebanese grapes and ochratoxin A in musts and finished wines during 2004. *J. Agric. Food Chem.* 2006, 54, 8977–8982. [CrossRef]  

11. Khoury, A.E.; Rizk, T.; Lteif, R.; Azouri, H.; Delia, M.L.; Lebrihi, A. Fungal contamination and aflatoxin B1 and ochratoxin A in Lebanese wine–grapes and musts. *Food Chem.* 2008, 109, 2244–2250. [CrossRef]  

12. Ordoñez, N.; García-Bastidas, F.; Laghari, H.; Ackary, M.; Harfouche, E.; Awar, B.A.; Kema, G.H. First Report of *Fusarium oxysporum* f. *sp. cubense* Tropical Race 4 Causing Panama Disease in Cavendish Bananas in Pakistan and Lebanon. *Plant Dis.* 2015, 100, 209. [CrossRef]  

13. Alloosh, M.; Hamwieh, A.; Ahmed, S.; Alkai, B. Genetic diversity of *Fusarium oxysporum* f. *sp. ciceris* isolates affecting chickpea in Syria. *Crop Prot.* 2019, 124, 104863. [CrossRef]  

14. Jimenez-Fernandez, D.; Navas-Cortés, J.A.; Montes-Borrego, M.; Jiménez-Díaz, R.M.; Landa, B.B. Molecular and pathogenic characterization of *Fusarium redolens*, a new causal agent of *Fusarium* yellows in Chickpea. *Plant Dis.* 2011, 95, 860–870. [CrossRef] [PubMed]  

15. Dean, R.; Van Kan, J.A.L.; Pretorius, Z.A.; Hammond-Kosack, K.E.; Pietro, A.D.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J.; et al. The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* 2012, 13, 414–430. [CrossRef]  

16. Munkvold, G.P.; Proctor, R.H.; Moretti, A. Mycotoxin Production in *Fusarium* According to Contemporary Species Concepts. *Ann. Rev. Phytopathol.* 2021, 59, 373–402. [CrossRef] [PubMed]  

17. Geiser, D.M.; Al-Hatmi, A.M.S.; Aoki, T.; Arie, T.; Balmas, V.; Barnes, I.; Bergstrom, G.C.; Bhattacharyya, M.K.; Blomquist, C.L.; Bowden, R.L.; et al. Phylogenomic Analysis of a 55.1-kb 19-Gene Dataset Resolves a Monophyletic *Fusarium* Species Complex. *Phytopathology* 2021, 111, 1064–1079. [CrossRef]  

18. Noorabadi, M.T.; Masiello, M.; Taherkhani, K.; Zare, R.; Torbati, M.; Haidukowski, M.; Somma, S.; Logrieco, A.F.; Moretti, A.; Susca, A. Phylogeny and mycotoxin profile of *Fusarium* species isolated from sugarcane in Southern Iran. *Microbiol. Res.* 2021, 252, 126855. [CrossRef] [PubMed]  

19. Rabaaoui, A.; Dall’Asta, C.; Righetti, L.; Susca, A.; Logrieco, A.F.; Namsi, A.; Gdoura, R.; Werbrouck, S.P.O.; Moretti, A.; Masiello, M. Phylogeny and Mycotoxin Profile of Pathogenic *Fusarium* Species Isolated from Sudden Decline Syndrome and Leaf Wilt Symptoms on Date Palms (*Phoenix dactylifera*) in Tunisia. *Toxins* 2021, 13, 463. [CrossRef]  

20. Castella, G.; Cabanes, F.J. Phylogenetic diversity of *Fusarium incarnatum-equiseti* species complex isolated from Spanish wheat. *Antonie Van Leeuwenhoek* 2014, 106, 309–317. [CrossRef]
21. Marin, P.; Moretti, A.; Ritieni, A.; Jurado, M.; Vazquez, C.; Gonzalez-Jaen, M.T. Phylogenetic analyses and toxigenic profiles of *Fusarium equiseti* and *Fusarium acuminatum* isolated from cereals from southern Europe. *Food Microbiol.* 2012, 31, 229–237. [CrossRef]

22. Logrieco, A.; Mulè, G.; Moretti, A.; Bottalico, A. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. *Eur. J. Plant Pathol.* 2002, 108, 597–609.

23. Desjardins, A.E. *Fusarium Mycotoxins: Chemistry, Genetics and Biology*; APS Press: St. Paul, MN, USA, 2006.

24. IARC (International Agency for Research on Cancer). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Human*; IARC: Lyon, France, 2002; Volume 82, pp. 1–556.

25. Jestoi, M. Emerging *Fusarium*-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin: A review. *Crit. Rev. Food Sci. Nutr.* 2008, 48, 21–49. [PubMed]

26. López-Berges, M.S.; Capilla, J.; Turrà, D.; Schafferer, L.; Matthijs, S.; Cornelis, P.; Guarro, J.; Haas, H.; Di Pietro, A. HapX-mediated iron homeostasis is essential for rhizosphere competence and virulence of the soilborne pathogen *Fusarium oxysporum*. *Plant Cell* 2012, 24, 3805–3822. [CrossRef] [PubMed]

27. Herrmann, M.; Zocher, R.; Haese, A. Effect of disruption of the enniatin synthetase gene on the virulence of *Fusarium avenaceum*. *Mol. Plant-Microbe Interact.* 1996, 9, 226–232. [PubMed]

28. Pérez-Hernández, A.; Rocha, L.O.; Porcel-Rodríguez, E.; Summerell, B.A.; Liew, E.C.Y.; Gómez-Vázquez, J.M. Pathogenic, morphological, and phylogenetic characterization of *Fusarium solani f. sp. cucurbitae* isolates from cucurbits in Almería Province, Spain. *Plant Dis.* 2020, 104, 1465–1476. [CrossRef]

29. Aoki, T.; O’Donnell, K.; Homma, Y.; Lattanzi, A.R. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex–*F. virguliforme* in North America and *F. tucumaniae* in South America. *Mycologia* 2003, 95, 660–684. [PubMed]

30. Nelson, P.E.; Toussoun, T.A.; Marasas, W.F.O. *Fusarium Species: An Illustrated Manual for Identification*; Penn State University Press: University Park, PA, USA, 1983.

31. Leslie, J.F.; Summerell, B.A. *The Fusarium Laboratory Manual*; Blackwell Publishing: Hoboken, NY, USA, 2006.

32. O’Donnell, K.; Cigelnik, E.; Nirenberg, H.I. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* 1998, 90, 465–493.

33. Edgar, R.C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 2004, 32, 1792–1797. [CrossRef]

34. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* 2016, 33, 1870–1874. [CrossRef]

35. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 1985, 39, 783–791. [CrossRef]

36. Edel-Hermann, V.; Lecomte, C. Current Status of *Fusarium oxysporum Formae Specialae* and Races. *Phytopathology* 2019, 109, 512–530. [CrossRef]

37. IARC (International Agency for Research on Cancer). *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans*; IARC: Lyon, France, 1993; Volume 56, pp. 445–466.

38. Sanzani, S.M.; Cariddi, C.; Roccotelli, A.; Garganese, F.; Fallanaj, F.; Ippolito, A. First report of *Gibberella avenacea* causing wet apple core rot in Italy. *J. Plant Pathol.* 2013, 95, 217.

39. Wenneker, M.; Pham, K.T.K.; Lemmers, M.E.C.; de Boer, F.A.; van der Lans, A.M.; van Leeuwen, P.J.; Hollinger, T.C.; Thomma, B.P.H.J. First report of *Fusarium avenaceum* causing wet core rot of ‘Elstar’ apples in the Netherlands. *Plant Dis.* 2016, 100, 1501. [CrossRef]

40. Laraba, I.; Keddad, A.; Boureghda, H.; Abdallah, N.; Vaughan, M.M.; Proctor, R.H.; Busman, M.; O’Donnell, K. *Fusarium algeriense*, sp. Nov., a novel toxigenic crown rot pathogen of durum wheat from Algeria is nested in the *Fusarium burgessii* species complex. *Mycologia* 2017, 109, 935–950. [CrossRef] [PubMed]