Chapter 1

Major and Emerging Fungal Diseases of *Citrus* in the Mediterranean Region

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Additional information is available at the end of the chapter

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Abstract

This chapter deals with major endemic and emerging fungal diseases of citrus as well as with exotic fungal pathogens potentially harmful for citrus industry in the Mediterranean region, with particular emphasis on diseases reported in Italy and Maghreb countries. The aim is to provide an update of both the taxonomy of the causal agents and their ecology based on a molecular approach, as a preliminary step towards developing or upgrading integrated and sustainable disease management strategies. Potential or actual problems related to the intensification of new plantings, introduction of new citrus cultivars and substitution of sour orange with other rootstocks, globalization of commerce and climate changes are discussed. Fungal pathogens causing vascular, foliar, fruit, trunk and root diseases in commercial citrus orchards are reported, including *Plenodomus tracheiphilus*, *Colletotrichum* spp., *Alternaria* spp., Mycosphaerellaceae, Botryosphaeriaceae, *Guignardia citricarpa* and lignicolous basidiomycetes. Diseases caused by *Phytophthora* spp. (oomycetes) are also included as these pathogens have many biological, ecological and epidemiological features in common with the true fungi (eumycetes).

Keywords: *Plenodomus tracheiphilus*, *Colletotrichum* spp., *Alternaria* spp., greasy spot, Mycosphaerellaceae, Botryosphaeriaceae, *Guignardia citricarpa*, Basidiomycetes, *Phytophthora* spp

1. Introduction

Citrus are among the ten most important crops in terms of total fruit yield worldwide and rank first in international fruit trade in terms of value. More than seven million hectares are planted with citrus throughout the world. The term "citrus" indicates a complex of species and hybrids of the genera *Citrus*, *Eremocitrus*, *Fortunella*, *Microcitrus* and *Poncirus*, subfamily

Aurantioideae (family Rutaceae). It is assumed that all presently cultivated citrus species originate from three ancestral "true" species, *Citrus medica* (citron), *Citrus reticulata* (mandarin) and *Citrus maxima* (pummelo). Although citrus are native to East Asia, citriculture has expanded in tropical, subtropical and Mediterranean climatic regions, and presently Mediterranean countries are the leading producers for the international fresh market. The term "fungal" diseases referred to citrus pathologies includes both diseases caused by "true" fungi or fungi sensu stricto (eumycota) and those caused by oomycetes. In fact, although Oomycetes are part of a distinct kingdom (Chromista or Stramenopiles), they are traditionally considered fungi sensu lato as they have in common with Eumycota some ecological and morphological (e.g. filamentous hyphae) features. This chapter is not intended to be a complete review of fungal diseases reported in the Mediterranean citrus belt. It deals only with major endemic and emerging fungal diseases of citrus reported in Italy and Maghreb countries, as well as with exotic fungal pathogens potentially harmful for citrus industry in the Mediterranean region. The aim is to provide an update of the taxonomy of the causal agents and their ecology and diagnosis based on a molecular approach as a preliminary step towards developing or upgrading integrated and sustainable management strategies. Postharvest fungal diseases of citrus are not within the scope of this brief review.

2. General considerations

Any rational disease management strategy is based on accurate diagnosis and prevention. Fungal diseases of citrus showing specific symptoms can be easily diagnosed visually, while for diseases with no typical symptoms, laboratory tests are needed. A limit of symptomatic diagnosis is that some symptoms are visible only at certain times of the year or appear on organs distinct from those colonized by the pathogen. Moreover, secondary parasites or opportunistic pathogens can overgrow primary pathogens or colonize senescent or necrotic tissues. Typical examples are *Colletotrichum* species such as the cosmopolitan *Colletotrichum gloeosporioides*, the most common *Colletotrichum* species on citrus and *Colletotrichum karstii. C. karstii* has recently been described as a separate species of the *Colletotrichum boninense* complex, using a multilocus molecular phylogenetic analysis [1]. It is a widespread and polyphagous species; however, so far it has been reported on citrus only in Italy and China [2, 3], very probably reflecting a sampling bias. *Colletotrichum* species are associated with citrus as endophytes, saprobes as well as pre- and postharvest anthracnose pathogens. They can switch their lifestyle from endophytes to saprobes or opportunistic pathogens and produce acervuli on necrotic tissues as a consequence of biotic and abiotic stresses, such as mal secco disease, frost, wind, hail and any type of mechanical injury.

The most effective control method of fungal diseases is prevention, especially for diseases caused by soil-borne pathogens. Most of the rootstocks used in commercial citrus orchards, e.g. are resistant to *Phytophthora* trunk gummosis and root rot. Genetic resistance has also been used in citriculture for prevention of vascular and canopy diseases such as mal secco disease of lemon and brown spot of tangerines, respectively. However, the choice of the scion is primarily conditioned by commercial requirements. The genetic susceptibility to fungal diseases is a limiting factor to the widespread diffusion of some citrus cultivars ['Fortune' mandarin, e.g. is not planted in humid areas as it is very susceptible to Alternaria brown spot (ABS)]. Prevention methods include selection of the plantation site, surface levelling of the ground to avoid waterlogging, soil drainage and rational management of irrigation. Irrigation systems that wet the trunk favour *Phytophthora* gummosis, but risk is reduced if trees are irrigated during the morning in order to allow the bark to dry quickly. Sprinkler irrigation under the canopy is conducive to *Phytophthora* infections on trunk and fruits, while overhead sprinkler irrigation favours brown spot epidemics in orchards of susceptible tangerine cultivars as well as *Septoria* spot and mal secco in lemon orchards. Generally speaking, localized irrigation methods, such as drippers, are less conducive to leaf and fruit diseases. Usually, fungal infections occur on citrus trees irrespective of their vigour. However some diseases attack only weakened trees, while others develop preferentially on vigorous trees. Alternaria brown spot, e.g. is more severe on trees with dense canopy and copious spring vegetation as the causal agent, *Alternaria alternata* (*A. alternata*), sporulates only on young leaves and with high relative humidity. As a consequence this disease is favoured by intensive plantings and high amounts of nitrogen fertilizers.

Mediterranean climate is not conducive to epidemic infections of fungal diseases of the tree canopy, and as a consequence, chemical control of these diseases in the Mediterranean region is economically justified only in few cases. The choice of fungicides is restricted to active ingredients registered for citrus. In Italy, only copper derivatives (oxychloride, hydroxide and sulphate tribasic) are allowed for field treatments against fungal diseases. Systemic fungicides, metalaxyl M and ethyl-phosphytes (Al ethyl-phosphite and K ethyl-phosphite) are available for chemical control of diseases caused by *Phytophthora*. Metalaxyl M can be applied as soil drench or trunk paint as it is translocated through the plant apoplast. The derivates of phosphorous acid are translocated through the symplast so they can be applied to the trunk (as paints or sprays) or the tree canopy (as leaf sprays). In some commercial products, cupric and systemic fungicides are blended together.

Hereafter we illustrate major fungal diseases of citrus already established or potentially harmful for the citrus industry in the Mediterranean region. Two quarantine fungal pathogens of citrus presently are included in the A1 list of the European and Mediterranean Plant Protection Organization (EPPO), *Guignardia citricarpa* (*G. citricarpa*) (anamorph, *Phyllosticta citricarpa*), the causal agent of fruit black spot, and *Pseudocercospora* (*Phaeoramularia*) *angolensis* (*P. angolensis*), the causal agent of angular leaf spot also known as Phaeoramularia leaf and fruit spot. However, only for *G. citricarpa* an official diagnostic protocol is available. The EPPO A1 list includes pathogens and parasites whose introduction in the territory of EPPO would cause severe phytosanitary risks. Import of citrus fruits and propagative material from areas where these pathogens are present is subject to customs restrictions. Geographic distribution of *G. citricarpa* includes Asia, Africa, Australia, South America and Florida. A real risk for the citrus industry in Italy is that this fungal pathogen can be imported with citrus from South Africa to South America. In the last years, this pathogen has been intercepted several times on citrus imported from South Africa, Brazil and Uruguay. For more details on citrus black spot, refer to the EPPO diagnostic protocol PM7/17 (http://www.eppo.int) [4]. The protocol reports official molecular diagnostic methods to identify the causal agent of black spot and distinguish it from the non-pathogenic species *Guignardia mangiferae*. *P. angolensis* causes leaf spots and necrotic lesions on fruits. Geographic distribution of this pathogen is restricted to the warm and humid areas of central Africa, at altitudes between 80 and 1500 m, and Yemen, in Asia. All species of cultivated Citrus appear to be susceptible.

3. Mal secco

The mal secco, an Italian name meaning "dry disease", is a vascular wilt disease (**Figure 1**) caused by the mitosporic fungus *Plenodomus tracheiphilus* (*P. tracheiphilus*), formerly *Phoma tracheiphila*. Symptoms include strands of salmon-pink to orange-red discoloration visible in stem xylem (**Figure 2**) as well as veinal chlorosis (**Figure 3**), wilt and shedding of leaves, dieback of twigs and branches. The disease is particularly destructive on lemon (*Citrus limon*) in Mediterranean countries and the Black Sea region. So far, however, it has not been reported in Spain, Portugal and Morocco, as well as other major citrus-growing regions of the world, even though there is no obvious climatic or cultural factor limiting its establishment in uninfested areas.

Figure 1. Wilting, leaf shedding and defoliation on a young lemon tree affected by mal secco (courtesy S.O. Cacciola, G. Magnano di San Lio, A. Pane).

Figure 2. Pink-salmon discoloration of the wood associated to mal secco disease (courtesy S.O. Cacciola and A. Pane).

Figure 3. Clearing and chlorosis of a sour orange leaf affected by *Plenodomus tracheiphilus* (courtesy S.O. Cacciola and A. Pane).

P. tracheiphilus is a quarantine pathogen of great concern to many international plant protection organizations; it is listed on the list A2 of EPPO and the lists of quarantine pathogens of Asia and Pacific Plant Protection Commission (APPPC), Caribbean Plant Protection Commission (CPPC), Comité Regional de Sanidad Vegetal del Cono Sur (COSAVE), North American Plant Protection Organization (NAPPO) and Inter-African Phytosanitary Council (IAPSC). Moreover *P. tracheiphilus* was included in a list of microorganisms that have to be regarded as potential biological weapons as they cause destructive diseases of economically relevant crops [5]. Although lemon is the principal host, other species of citrus and related genera (*Fortunella*, *Poncirus* and *Severinia*) may be infected [6]. In several Mediterranean countries, including Greece, Israel, Italy and Tunisia, severe infections have been sporadically observed, also in commercial orchards of bergamot, citron, sweet oranges, tangerines, mandarins and mandarin hybrids. Many of these reports on sweet oranges, tangerines, mandarins and mandarin hybrids refer to the chronic *facies* of the disease known as "mal nero" (**Figure 4**), very probably originating in the nursery. Young seedlings of common citrus rootstocks such as sour orange (*Citrus aurantium*), citranges (*Citrus sinensis* 'Washington' sweet orange × *Poncirus trifoliata*) and citrumelo 'Swingle' (*Citrus paradisi* 'Duncan' grapefruit × *P. trifoliata*) proved to be susceptible to natural infections in nursery.

Figure 4. The 'mal nero' *facies* of mal secco disease (courtesy S.O. Cacciola, G. Magnano di San Lio, A. Pane).

Alemow (*Citrus macrophylla*) is extremely susceptible, and trees on alemow rootstock may die as a consequence of root infections. This *facies* of the disease associated to root infections is known as "mal fulminante", an Italian name meaning "sudden death".

The mal secco fungus was originally classified among the Deuteromycota as *Deuterophoma tracheiphila*. Later, it was reclassified as *P. tracheiphila* and considered a member of the subgenus *Plenodomus* due to the presence of thick-walled cells in the pycnidial scleroplectenchymatous tissues [7]. Many species in this subgenus have *Leptosphaeria* teleomorphs and/or *Phialophora* synanamorphs [8]. Although a molecular phylogenetic study has shown a relationship between *P. tracheiphila* and *Leptosphaeria* species [9], the teleomorph of the mal secco fungus has not yet been identified.

Recent molecular phylogenetic studies on the genus *Phoma* demonstrated its polyphyly and lead to the definition of the genus *Plenodomus*. *P. tracheiphila* and many other fungi of the section *Plenodomus* were allocated within this genus [10–12]. Presently, therefore the accepted scientific name of the causal agent of mal secco disease of citrus is *P. tracheiphilus*. Identification of *P. tracheiphilus* is currently based on morphological and molecular methods as described in the OEPP/EPPO diagnostic protocol PM7/048(3) (http://www.eppo.int) [13]. In the last years, numerous molecular techniques based on conventional polymerase chain reaction (PCR) and real-time PCR (rtPCR) have been developed, allowing fast and sensitive detection and quantification of the pathogen from infected tissues [9, 14–17]. Some of these techniques have been applied in breeding programmes to monitor the progress of the fungus in the host xylem and test the susceptibility of lemon cultivars to mal secco. As in other tracheomycoses, in fact, there is a direct correlation between the rate and extent of xylem colonization by the pathogen (i.e. the amount of the fungus in the vessels) and the susceptibility of the cultivar to the disease as determined by symptom severity.

P. tracheiphilus can infect citrus hosts all year round, but most infections occur from September to April. Pycnidiospores produced within pycnidia and phialoconidia produced on mycelium are the infective propagules. Pycnidia are present throughout the year on withered twigs (**Figure 5**) and branches; pycnidiospores are extruded when the weather is wet. Phialoconidia are produced on the surface of infected tissues exposed by wounds or on woody debris in soil. Penetration occurs through wounds. Penetration through natural opening has been hypothesized but never demonstrated. After penetration, the mycelium grows in the lumen of xylem vessels producing both phialoconidia and blastoconidia that are translocated by the ascendant lymph and invade the wood. The pathogen can be detected distantly from its penetration site, by molecular tools, before the expression of the disease symptoms [17]. Systemic invasion by the pathogen leads to the loss of xylem functionality and the appearance of water stress symptoms typical of tracheomycoses. Impairment of water transport is proved by the increase of hydraulic resistance of the stem and leaves as well as the closure of stomata [18, 19]. Although the optimum temperature for pathogen growth is about 25°C, optimum temperature for symptom expression and xylem colonization is 20–22°C.

Infection occurs between 14 and 28°C, whereas at temperatures above 28°C, fungal growth ceases and symptoms are not expressed. As a consequence disease progress is temporarily inhibited during the hot or cold temperature extremes.

Figure 5. White-red 1–2-year-old twig of lemon with pycnidia of *Plenodomus tracheiphilus*. Pycnidia appear as scattered black spots on the dried portion of the twig (courtesy S.O. Cacciola and A. Pane).

While in Sicily most infections occur from autumn to early spring; in Israel, mid-November to mid-April was the most conducive time for infection, coinciding with the rainy season, although no correlation was found between the amount of rain, the number of rainy days and the percentage of infected plants. No infection was observed after the rain ceased, so it appears that the rain affects inoculum dissemination rather than infection [20]. Length of the incubation period varies according to season, and in young trees, it ranges between 2 and 7 months, whereas it can last several years in the "mal nero" form of the disease because this chronic infection could remain confined to the heartwood over a long time. Expression of symptoms is therefore a poor selection criterion for phytosanitary inspection of propagation material. This aspect has practical relevance as the use of disease-free propagation material helps to reduce the dissemination of mal secco and its introduction into disease-free areas.

Like for other tracheomycoses, fungicide treatments are not effective against mal secco, and research of newly resistant genotypes remains the only effective strategy to control this disease. Lemon cultivars with various degrees of resistance to mal secco, such as 'Monachello', 'Interdonato', 'Feminello Zagara Bianca', 'Femminello Continella' and 'Cerza', have been selected in Sicily. However, 'Monachello' has a poor yield, 'Interdonato' does not bloom several times, and its juice has low acidity, and the tolerance to mal secco of the other cultivars is not comparable to that of 'Monachello'. Two new cultivars with high yield potential, 'Femminello Siracusano 2Kr', a mutant nucellar clone obtained with cobalt γ-radiation, and the triploid hybrid 'Lemox' (European patent number 20040073), have been included in the official list of lemon cultivars whose use is recommended in Italy for new plantings. However they proved to be extremely susceptible to mal secco disease. The goal of obtaining tolerant cultivars with competitive yields and satisfactory bio-agronomic characteristics remains one of the primary objectives of lemon-breeding programmes in the Mediterranean region. Additional and more detailed information on mal secco disease can be found in two recent comprehensive reviews [20, 21].

4. Emerging and endemic foliar and fruit diseases

Despite the Mediterranean climate is not conducive to epidemic outbreaks of fungal diseases of the canopy of citrus trees, being characterized by long periods of drought and high temperatures in summer as well as cool winters, in the last years, some citrus-growing areas of the Mediterranean region have experienced the emergence or resurgence of new and endemic fungal diseases of leaves and fruit.

4.1. Alternaria brown spot

Alternaria brown spot (ABS) is one of the most important diseases of tangerines and their hybrids worldwide. It is caused by the tangerine pathotype of the fungus *A. alternata* [22, 23]. *A. alternata* is a typical necrotrophic pathogen. It produces a host-specific (hs) toxin named ACT-toxin (ACTT), which induces necrotic lesions on fruit and young leaves, defoliation and fruit drop in susceptible citrus genotypes. There are several pathotypes of *A. alternata* characterized by host specificity [24]. The chemical structure of ACT-toxin is similar to those of other hs-toxins such as AK- and AF-toxin, produced by the Japanese pear and strawberry pathotypes, respectively [25, 26]. The tangerine pathotype of *A. alternata* carries a gene cluster (ACTT) located in a small chromosome which is responsible for the biosynthesis of ACT-toxin [27]. There is also indirect evidence suggesting the presence of toxin receptors in susceptible citrus genotypes. In addition, recent studies confirm that the ACT-toxin is a pathogenicity factor and indicate that the mitigation of reactive oxygen species (ROS) produced by the host plant is essential for the pathogenicity of *A. alternata*. A significant correlation was found between pathogenicity on citrus leaves and ACTT gene expression in isolates of *A. alternata* from citrus of various geographic origins [28]. ACT-toxin is released during the germination of conidia. It induces necrotic areas of the leaf blade and may be translocated to the vascular system inducing chlorosis and necrosis along the veins.

ABS is prevalent in citrus production areas with a Mediterranean climate, characterized by cool, humid winters and hot, arid summers. It was first reported on 'Emperor' mandarin in Australia in 1903, and subsequently it was detected in the Americas, the Mediterranean basin, South Africa, Iran and China affecting mainly 'Fortune' and 'Nova' mandarin hybrids [22, 29, 30]. In Europe, it has been reported in Greece, Italy and Spain. Its appearance in Italy coincided with the diffusion of the mandarin 'Fortune'. Warm temperatures and prolonged wetness are required for infection. However the disease causes severe epidemics in both humid areas and semi-arid regions provided a susceptible citrus variety is present. Fruits can get infected in all development stages but are more susceptible during the first four months following petal fall. Spring infections on young fruits may lead to premature fruit drop. Early fruit drop is common, especially if infection has occurred shortly after petal fall. Symptoms on fruits are necrotic brown circular lesions that may vary in size (**Figures 6** and **7**). Mature

Figure 6. Typical symptoms of Alternaria brown spot on 'Fortune' mandarin (courtesy S.O. Cacciola, G. Magnano di San Lio, A. Pane).

Figure 7. Alternaria brown spot on 'Nova' tangelo hybrid (courtesy S.O. Cacciola, G. Magnano di San Lio, A. Pane).

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lesions have a corky appearance, and in older lesions, the centre may dislodge leaving tan-coloured pockmarks. Brown to black lesions surrounded by yellow halos and veinal necrosis appear on young leaves, which often are deformed due to necrosis of the margin. On highly susceptible cultivars abundant defoliation, abscission of young shoots and twig dieback may occur. Conditions of persistent humidity (fog or dew), which provide a wetting period of 8–12 h, are conducive for the development of infections; the optimum temperature is 20–27°C, but infections can occur between 17 and 32°C. The disease incubation period is 16–36 h. Conidia are produced on necrotic lesions in young leaves but not on fruits and are dispersed by air currents and rain splash. The presence of the disease is a limiting factor for the diffusion of highly susceptible mandarin or tangerine-like cultivars such as 'Fortune', 'Dancy', 'Minneola', 'Orlando', 'Nova', 'Guillermina', 'Clemenpons', 'Esbal', 'Page', 'Lee', 'Sunburst', 'Encore', 'Murcott', 'Michal', 'Winola', 'Ponkan', 'Emperor', 'Tangfang' and 'Primosole'. Even some varieties of pomelo are susceptible, while orange cultivars, with very few exceptions, are resistant.

Lemon and lime cultivars are not susceptible, with the exception of Mexican lime (*Citrus aurantifolia*) which is slightly susceptible.

Generally speaking, hybrids with 'Dancy' and 'King' mandarins as a parent are very susceptible. In many countries, such as Italy, Israel, Spain and the USA, ABS is a strong concern for triploid breeding programmes aiming at producing seedless mandarin cultivars. From diploid progeny analysis, it has been proposed that the inheritance of ABS susceptibility in citrus is controlled by a single gene with two alleles, one dominant (S) and the other recessive (r) which transmit susceptibility and resistance, respectively [31]. Therefore, resistant cultivars are considered to be recessive homozygous for this locus, whereas susceptible cultivars could be heterozygous or homozygous dominant. Cultivars like 'Minneola' and 'Dancy', which are homozygous (SS), transmit susceptibility to all the descendants. Most susceptible commercial cultivars like 'Fortune', 'Nova' and 'Murcott' are heterozygous, and both resistant and susceptible hybrids can be found in their progeny.

Resistant oranges, mandarins and clementines are recessive homozygous (rr), so when they breed with each other, all descendants are resistant. The single-locus dominant inheritance of susceptibility was corroborated by the analysis of triploid progenies. Recently, in Spain two new ABS-resistant hybrids of 'Fortune', 'Garbi' ('Murcott' × 'Fortune') and 'Safor' ('Kara' × 'Fortune') have been released.

Currently on susceptible cultivars, ABS control is based on the application of fungicides [32, 33]. Sprays must be scheduled to protect susceptible organs during the critical period for infection. Depending on the climate and the susceptibility of the cultivar, between four and ten fungicide sprays per year may be needed to produce quality fruit for the fresh market. On susceptible cultivars, foliar applications with copper fungicides are requested every 10–15 days in periods of high susceptibility. Despite this large number of sprays, disease control is not always satisfactory, and cultivation of very susceptible cultivars such as 'Fortune' in Mediterranean countries and 'Minneola' in Florida has declined significantly.

An integrated approach can reduce the risk of ABS infections and the disease severity [22]. In the nursery, it is recommended to grow susceptible citrus cultivars indoors, to avoid infections on young shoots and prevent inoculum dissemination in new commercial citrus plantings. New plantings of susceptible cultivars should be established in ventilated sites where environmental conditions are unfavourable for infections and sporulation of the causal agent on young leaves. Similarly, dense planting is not recommended for susceptible cultivars. Orchards of susceptible cultivars should be monitored frequently to detect the presence and prevent epidemic outbreaks of the disease.

4.2. *Septoria* spot

Septoria is a genus of plant pathogenic fungi with a wide geographic distribution, commonly associated with leaf spots and stem cankers of a broad range of host plants. Species of *Septoria* are among the most common and widespread leaf-spotting fungi worldwide. The causal agent of *Septoria* spot of citrus has been identified as *Septoria citri* [34]. This disease has been found in many citrus-producing countries of the Mediterranean basin, South Africa, South America, Australia (including Tasmania, Eastern and Southern Australia), India and California. Lemon (*C. limon*) and grapefruit (*C. paradisi*) are the most frequently damaged *Citrus* species worldwide, but all commercial citrus cultivars are susceptible. In Australia grapefruit, lemon and sweet orange (*C. sinensis*) 'Washington navel' are regarded as the most susceptible hosts. 'Valencia' oranges for juice production can also be affected although this cultivar is considered less susceptible than 'Washington Navel'. In California, Septoria spot affects 'Valencia' oranges, late-season navel oranges and occasionally lemons and grapefruits. It occurs in the San Joaquin Valley and interior districts of southern California during cool, moist weather. In Italy, *Septoria* spot has been reported on lemon, clementine and bergamot. Surprisingly, *S. citri* is a quarantine organism for Western Australia and South Korea. In 2004, Korean National Plant protection and Quarantine Service (NPQS) detected and rejected citrus fruits infected with Septoria spot imported from California. In most citrus-producing countries, *Septoria* spot is generally considered a disease of minor significance, except for fruit produced for the fresh market as rind blemishes reduce fruit quality aesthetically and affect saleability. Symptoms on fruit are small (1–2 mm in diameter), round, light tan-coloured lesions (pits) with a narrow green margin on the outer rind. As the fruit matures, they become reddish to pale brown (**Figure 8**) and contain small black spots (*S. citri* pycnidia) barely visible to the naked eye. When frost occurs or during storage fruit, lesions may enlarge (3–10 mm in diameter) and merge to form brown-to-black sunken blotches. These may be several centimetres in diameter and extend to the inner rind (albedo) and occasionally into the fruit segments. In severe infections, fruits develop an off flavour and drop prematurely. Symptoms may not appear until fruit is in storage. Leaf symptoms incited by *S. citri* are initially confined to the lower surface of the leaf and consist of small, blister-like brown to black spots, 1–4 mm in diameter, surrounded by a yellow halo. After leaf fall the spots turn brown with a dark margin and the pycnidia of the fungus form on necrotic tissues. Where under-canopy irrigation is used, infection may result in severe defoliation of the lower part of the tree (canopy skirt).

Septoria spot is more severe in years when rainfall levels are high and temperature fluctuates. The causal agent survives in infected orchards as a saprobe. Inoculum is constituted by pycnidia forming on dead twigs and leaves. Conidia, the infective propagules, are dispersed by water splash. Infections usually occur during cool, damp weather in late summer or autumn and when the fruits are still green. They may remain latent for up to six months, and fruit symptoms generally appear as the fruit starts ripening in late winter and early spring, after

Figure 8. Typical symptoms of *Septoria* spot on a lemon fruit (courtesy S.O. Cacciola and A. Pane).

cool, frosty or cold windy weather. The susceptibility of fruits is related to the maturity of the rind at the time of infection. Management practices to prevent or reduce the disease incidence and severity include tree skirting and canopy pruning to improve air circulation, early fruit harvesting and the removal of withered twigs from the tree canopy and fallen leaves from the soil under the tree canopy to reduce the amount of inoculum. Copper sprays in late fall or early winter to control fruit brown rot caused by *Phytophthora citrophthora* are also effective against *Septoria* spot.

The traditional taxonomy of *Septoria*, accommodating more than 2000 species, is confused as it has been based on few and conserved morphological characters. Moreover, it has been largely dependent on host data, and most species are not restricted to a single host. However, during the last years, the taxonomy of *Septoria* has been revisited using a polyphasic approach including both multilocus DNA sequencing and morphological characters. A more robust classification system is now available [35, 36]. In view of the worldwide distribution of *S. citri* and its status as a quarantine pathogen in some countries, it would be interesting to examine the genetic variability of *Septoria* populations associated to *Citrus* in different citrus-growing areas of the world in the frame of this new classification system.

4.3. Greasy spot and other cercosporoid diseases

Several species of cercosporoid fungi have been associated with leaf and fruit spot diseases of *Citrus* spp. Two of these diseases are particularly serious, Greasy spot, caused by *Zasmidium citri (Z. citri)-griseum* (sexual morph *Mycosphaerella citri*), and Phaeoramularia fruit and leaf spot, caused by *P. angolensis*, a fungus of quarantine concern for the European and Mediterranean Region.

Symptoms of greasy spot appear as yellow to dark brown to black lesions occurring first on the underside of mature citrus leaves. As the lesions develop on the underside of the leaves, they become darker, and a corresponding chlorotic spot appears on the upper leaf surface. Symptoms differ among citrus species. On highly susceptible species, such as lemon, spots are diffuse and tend to remain yellow, while on grapefruit, which is somewhat less susceptible, lesions are less diffuse, more raised and darker. On mandarins and 'Valencia' oranges, which are much more tolerant, lesions are smaller, brown to black and much more raised. Affected leaves fall prematurely from the tree during the fall and winter, resulting in reduced tree vigour and yield. Beside defoliation, the disease causes a rind blemish on fruit which has been referred to as greasy spot rind blotch. Greasy spot rind blotch significantly reduces the marketability of fruit for fresh consumption and is a serious problem especially on grapefruit but can also occur on oranges and other citrus. Greasy spot was first reported in Florida and is now endemic in all citrus-growing areas of the Caribbean Basin [37]. It also occurs in Texas but does not cause serious damage, probably because of a drier climate. Similar diseases of citrus have been observed in Argentina, Australia, China, Brazil, Egypt, Japan, Korea, Morocco, Spain and Italy. However the causal agent is not always *Z. citri-griseum* or has not identified. In a recent study, four *Zasmidium* species have been recognized on *Citrus*, namely, *Z. citri-griseum*, which has a worldwide distribution and a wide host range, and the three Asian species *Zasmidium fructicola*, *Zasmidium fructigenum* and *Zasmidium indonesianum* [38].

During the last years, an epidemic outbreak of a foliar disease closely resembling greasy spot has been observed in some citrus-growing areas of western Sicily (Italy). Symptoms appear on mature leaves and range from those typical of greasy spot (**Figures 9** and **10**) to black dots. Premature leaf drop occurs and causes heavy defoliation of the tree.

Figure 9. Symptoms of foliar greasy spot on the upper leaf surface of sweet orange (courtesy S.O. Cacciola and A. Pane).

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Figure 10. Symptoms of foliar greasy spot on the lower leaf surface of sweet orange (courtesy S.O. Cacciola and A. Pane).

The analysis of the fungal community using an amplicon metagenomic approach has revealed that Mycosphaerellaceae were the dominant group of fungi, in both symptomatic and asymptomatic leaves, and were represented by the genera *Ramularia*, *Mycosphaerella* and *Septoria*, with about 44, 2.5 and 1.7% of the total detected sequences [39]. The most abundant sequence type was associated to *Ramularia brunnea*, a species originally described to cause leaf spot in a plant of the family Asteraceae. Surprisingly, none of the detected sequences clustered with reference species currently reported as possible causal agents of greasy spot. Results are not conclusive and the aetiology of this emerging disease is still unresolved.

5. Bleeding cankers caused by Botryosphaeriaceae and *Phomopsis/Diaporthe*

Botryosphaeriaceae and *Phomopsis/Diaporthe* spp. are known to cause cankers on a variety of woody hosts including citrus. Formerly this disease of citrus was known as Dothiorella canker or Dothiorella gummosis because the pathogens most often isolated were *Dothiorella* spp. However, recent studies have shown that the disease is caused by a complex of fungal species, of which the most common belong to the family Botryosphaeriaceae and to a lesser extent the genera *Phomopsis/Diaporthe*.

On citrus trees, cankers are found prevalently on trunk and main branches. The canker exudes a reddish gum, giving it a bleeding, water-soaked appearance. Symptoms may also include wilt of shoots and branches, sometimes with dead leaves still attached. Two types of fruiting bodies (perithecia and pycnidia) can be found on cankers, which are the sexual and asexual stage of these fungi, respectively. They produce the infective spores and appear as tiny black bumps protruding from the bark. Pycnidial spores that are far more frequently observed in nature than perithecial spores ooze out in a ribbon-like gelatinous matrix and are usually disseminated by rain splash. Botryosphaeriaceae and *Phomopsis*/*Diaporthe* gain entrance into the host through both wounds and natural gaps in bark continuity.

Species of Botryosphaeriaceae (*Botryosphaeriales*, *Dothideomycetes*) are cosmopolitan. Most of them have a wide host range. On citrus, like other fruit trees, Botryosphaeriaceae are found especially on stem and woody branches. In recent studies, the taxonomy of this group of fungi has been radically revised using multigene phylogenetic analysis, and presently the family comprises 22 recognized genera [40], including *Diplodia*, *Botryosphaeria*, *Neofusicoccum*, *Dothiorella*, *Neoscytalidium*, *Macrophomina*, *Lasiodiplodia* and *Sphaeropsis*, just to cite a few of them. Also the taxonomy of *Diaporthe* and its asexual morph *Phomopsis* has been revised on the basis of phylogenetic and molecular data, and new species associated as endophytes with citrus have been described [41, 42]. The report as a new disease of shoot blight, associated with sooty cankers and gummosis, caused by *Neoscytalidium dimidiatum* in top-worked 'Tarocco Scirè' trees on sour orange rootstock is an example of the radical change in classification system and nomenclature of these groups of fungi. The same disease, in fact, was already known as Hendersonula branch wilt as the causal agent was originally identified as *Hendersonula toruloidea* [43]. Botryosphaeriaceae and *Phomopsis*/*Diaporthe* spp. are associated with citrus not only as endophytes but also as saprobes as well as latent pathogens.

Biochemical and genetic stimuli, resulting from environmental changes inside the hosts (changes in host physiological conditions or microbial equilibrium) or outside the host (climatic changes or extreme environmental events), trigger these fungi to change their lifestyle from endophytic to pathogenic. Therefore these fungi can be regarded as opportunistic fungal pathogens, and the management of diseases they cause is based essentially on preventing environmental stresses. In particular, an agronomical means to prevent the disease is to avoid water stress by reducing the time intervals between irrigations. Surgical removal of infected bark does not restrict the expansion of cankers, and copper treatments are only partially effective to reduce the inoculum on the tree. They can be recommended to protect top-worked stumps and prevent shoot blight.

6. Wood rots

Wood rots are caused by a wide variety of wood-degrading microorganisms and are characterized by decay and discoloration of wood of the trunk, large branches and main roots. Most wood-degrading fungi are Basidiomycetes that on living trees can cause two major kinds of decay: brown and white rots. Although wood-decay fungi play an ecologically important role as primary biotic decomposers of wood in forest ecosystems, they can cause economic losses in cultivated orchards by contributing to the premature ageing and the structural failure of the trees. It can also affect young trees as a result of abiotic stress, such as severe frosts and sun burning of branches exposed by heavy pruning. Most wood-decay fungi penetrate through wounds, although a few of the root-infecting species can enter the unwounded surface directly.

Citrus wood rot is a chronic disease occurring endemically on old trees in most citrus-growing areas of the world. Although this disease is not a major constraint for the citrus industry, it can

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contribute to the deterioration of orchards because affected trees show a premature ageing, a progressive decline in vigour and reduced productivity. A direct effect of wood decay is the breakage of scaffold branches due to loss of wood strength. Moreover trees show symptoms of leaf chlorosis and twig dieback. The incidence of the disease is high in more than 40-year-old orchards, and its severity depends on environmental conditions and susceptibility of the citrus species and cultivars. In particular, lemon trees are significantly more susceptible to wood decay than other types of citrus, including orange, grapefruit and tangelo. In addition, the disease incidence seems to be correlated with the intensity and pattern of precipitations. As far as the Mediterranean region is concerned, *Fomitiporia mediterranea* (*F. mediterranea*) was found to be the most common white wood-rotting fungus of citrus [44–46], whereas *Fomitopsis* sp. was associated with brown rot [47]. Other nonidentified basidiomycetous fungi showing genetic affinity with *Phellinus* and *Coniophora* were occasionally recovered from decayed wood.

F. mediterranea is a ubiquitous and polyphagus species, commonly found also on other fruit, ornamental and forest tree species, such as hazelnut, olive, kiwi, locust tree and privet. On grape, it is associated to 'Esca' disease. It is assumed that most infections in citrus orchards originate from airborne basidiospores germinating on large pruning wounds of trunk and main branches. Basidiospores produced by basidiomata germinate with relative humidity over 90% and are dispersed by wind. Usually, basidiomata (**Figure 11**) emerge from the bark after the wood of trunk or branches has been extensively colonized by the fungus (**Figure 12**). In Southern Italy, the analysis of *F. mediterranea* internal transcribed spacer (ITS) sequences revealed a high level of genetic variability, with both homozygous and heterozygous

Figure 11. Symptoms of wood rot caused by *Fomitiporia mediterranea* on *Citrus* sp. (courtesy S.O. Cacciola and A. Pane).

Figure 12. Basidiocarp of *Fomitiporia mediterranea* (courtesy S.O. Cacciola and A. Pane).

genotypes. This and the high frequency of basidiomata in old commercial orchards confirm the outcrossing nature of reproduction in *F. mediterranea* and the primary role of basidiospores in the dissemination of inoculum. Prevention is the only way to manage wood rots. Trees should be kept healthy and vigorous. Large pruning cuts, or other wood-exposing injuries, should be avoided, especially during wet periods. Proper management guidelines include sanitation of pruning cuts with mastics to prevent the penetration of the pathogen.

7. *Phytophthora* diseases

The all-inclusive term "*Phytophthora* diseases" indicates a complex pathology which is caused by soilborne species of *Phytophthora* and is recognized as a major fungal disease of citrus almost universally. *Phytophthora* species attack citrus plants at all stages and may infect all parts of the tree, including roots, stem, branches, twigs, leaves and fruits. There are several forms (*facies*) of *Phytophthora* diseases including root rot, foot rot (**Figure 13**) (also known as *Phytophthora* gummosis, trunk gummosis or collar rot), fruit brown rot (**Figure 14**), twig and leaf dieback (often indicated collectively as canopy blight) and rot of seedlings (better known as damping off of seedlings). Trunk gummosis and root rot are the most serious *facies* of this group of diseases, and after the pandemic outbreak of the nineteenth century and the consequent widespread use of resistant rootstocks, they are regarded as endemic diseases in all citrus-growing areas of the world.

Figure 13. Trunk gummosis caused by *Phytophthora citrophthora* on a citrus tree (courtesy S.O. Cacciola and A. Pane).

At least ten species of *Phytophthora* have been reported to attack citrus in the world, but the commonest species in commercial citrus orchards of the Mediterranean region are *P. citrophthora* and *P. nicotianae* [48]. The latter is the dominant species and is usually associated to root rot, while *P. citrophthora* is frequent in old plantings and is commonly associated to trunk

Figure 14. Sweet orange fruit with symptoms of brown rot caused by *Phytophthora citrophthora* (courtesy S.O. Cacciola and A. Pane).

gummosis. Other species found occasionally include *Phytophthora citricola*, *Phytophthora cactorum*, *Phytophthora hibernalis* and *Phytophthora syringae*. The last two species are found during winter months solely because of their low-temperature requirements.

Recently, *Phytophthora meadii*, which is new for the Mediterranean region, was recorded in rhizosphere soil of potted ornamental citrus plants in southern Italy, using a very sensitive amplicon metagenomic approach with genus-specific primers [49, 50]. Both *P. citrophthora* and *P. nicotianae* are polyphagous.

Interestingly, however, genetic analyses of a worldwide collection of *P. nicotianae* isolates from different hosts, including agricultural crops and ornamentals, revealed that isolates from citrus clustered together and constituted a separate group regardless of their geographic origin [51–53]. This result is indicative of host specialization and suggests that *P. nicotianae* population associated to citrus has been very likely spread worldwide with infected nursery plants.

Temperature is a major ecological factor affecting seasonal fluctuations of *P. citrophthora* and *P. nicotianae* and their distribution. *P. nicotianae* prefers warmer temperatures, and in the Mediterranean region, it is not active in winter, producing chlamydospores which allow the pathogen to survive in unfavourable conditions. By contrast, *P. citrophthora* is not inhibited by low temperatures, and during fall and winter, it can cause brown rot outbreaks. Another epidemiological difference between the two species is the ability of *P. citrophthora* to produce sporangia on fruits, which are the sources of the secondary inoculum. This ecological feature explains sudden epidemic explosions of brown rot, following persistent rainfall. Neither of the two species forms sporangia on the gummy cankers at the base of the trunk. As far as it is known, *P. citrophthora* does not reproduce sexually, and *P. nicotianae* reproduces sexually only occasionally, since only A1 mating type is found in most citrus orchards.

Sporangia produced in the most superficial soil layer (0 to about 30 cm depth), on contact with air, are the main source of inoculum. Natural infections are most frequently caused by zoospores and occasionally by direct or indirect germination of sporangia through a germ tube or by releasing zoospores, respectively. Production and germination of sporangia are influenced by temperature and soil water potential. Their dissemination is mostly by water splash and occasionally by wind, within water droplets. The zoospores are motile and can swim short distances by flagellar movement or can be carried over longer distances by soil water. They swim towards roots, as they are attracted by root exudates, and encyst upon contact, germinate and penetrate fruits, leaves, shoots and green twigs directly.

Grafting on resistant rootstocks, such as sour orange, is the most practical and widely used means to control *Phytophthora* gummosis. The rootstocks that are substituting sour orange in the Mediterranean region, following the epidemic spread of *Citrus tristeza virus* (CTV), to which sour orange is very susceptible, are mainly citranges, hybrids of trifoliate orange (*P. trifoliata*) and sweet oranges, such as 'Troyer', 'Carrizo' and 'C-35' citranges. Generally speaking, rootstocks resistant to *P. citrophthora* and *Phytophthora* gummosis are also resistant to *P. nicotianae* and root rot, with few exceptions (e.g. 'Carrizo' citrange is resistant to *Phytophthora* gummosis but susceptible to root rot, while trifoliate orange is resistant to both *facies* of the disease).

Brown rot is both a preharvest and postharvest decay of citrus fruit. Infected fruit shows a typical leathery brown rot with indistinct edges and has a characteristic rancid smell. With high moisture in the environment, white furry mould forms on the fruit surface. Infections cause the fruit to drop prematurely and occur especially on fruits hanging in the lower part of the tree canopy, with rain splash. Epidemic explosions are more frequent in citrus orchards where trunk gummosis is endemic. The incubation period of the disease is 7–10 days at 10°C but may be longer with lower temperatures. Asymptomatic infected fruits can infect healthy fruits even after harvesting, during transportation and storage.

Two major breakthroughs in the implementation of integrated management strategies of *Phytophthora* diseases were the launch of systemic fungicides with specific activity against Oomycetes [54–58] and the development of selective media for direct isolation of *Phytophthora* spp. from soil and infected tissues. Serial dilutions of soil suspensions on a selective medium became a very popular method for monitoring the amount of *Phytophthora* inoculum in the soil. Monitoring was used to study seasonal fluctuations of *Phytophthora* populations to manage the irrigation as well as to schedule chemical treatments and evaluate their effects [59–62]. The rationale of these strategies is the assumption that a direct correlation exists between the amount of inoculum and the incidence and severity of root rot. Although a very sensitive molecular method based on real-time PCR with specifc primers was developed for the detection of *P. citrophthora and P. nicotianae* in soil [63], soil dilution on a selective medium in Petri dishes still remains the preferred and most widely used standard method for quantitative determination of *Phytophthora* inoculum. The quantity of inoculum determined with this microbiological method is expressed in terms of colony-forming units (CFU)/g or cm^3 of soil. The threshold intervention level in bearing citrus orchards is between 10 and 30 CFU/cm^3 of soil, but ideally a zero-tolerance threshold would be requested for nursery stocks to be sold. More details on *Phytophthora* diseases may be found in many comprehensive reviews [48, 64–66].

8. Concluding remarks

Over the last decade, there have been a number of publications dealing with molecular studies on fungal pathogens of citrus, focusing particularly on their identification and genetic diversity. In particular, new species of *Colletotrichum*, Botryosphaeriaceae and Mycosphaerellaceae have been described, and significant progress towards a new, unified phylogenetic classification system of these groups of fungi has been achieved, for which substantial advances were obtained by multigene sequencing and phylogenetic analysis. However, there have been fewer publications related to other molecular aspects such as the mechanisms underlying the host-pathogen interactions. With the development of next-generation sequencing techniques and the availability of whole-genome sequences, molecular studies are expected to get insight into the biology, pathogenicity and ecology of well-identified pathogens as well as the aetiology of diseases whose causal agent is still undetermined. Moreover, diversity studies will provide epidemiological information such as distribution, virulence and genetic structure of pathogen populations. The results of preliminary applications of these new molecular techniques to the study of citrus diseases, such as *Phytophthora* diseases and greasy spot-like diseases, are very promising.

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