Chapter 17

Diseases caused by fungi and oomycetes

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17.1 Greasy spot disease of citrus caused by *Zasmidium citri-griseum*

17.1.1 Introduction

Zasmidium citri-griseum (= *Mycosphaerella citri*) belongs to the *Mycosphaerellaceae* family and is the causal agent of the greasy spot (Whiteside, 1970). *Z. citri-griseum* infects citrus inducing leaf spots, premature defoliation, which results in reduced fruit size, fruit drop, and yield loss (Aguilera-Cogley et al., 2017). Rind blotch occurs when the pathogen directly infects the fruit. Greasy spot is widespread in the southeast North America (Florida and Eastern Mexico), Central America, Northern South America, and the islands of the Caribbean (Timmer and Gottwald, 2000). Outside the American continent, Japan, Egypt, and China have also reported greasy spot in citrus to be caused or associated with this same species (Leki, 1986; Haggag, 2012; Huang et al., 2015b). Similar greasy spot-like symptoms have been reported on citrus in other countries, however, this was associated with different *Mycosphaerellaceae* species (Huang et al., 2015b; Aguilera-Cogley et al., 2017).

17.1.2 Disease symptoms

Leaf tissues infected with *Z. citri-griseum* show chlorosis and yellow-brown pustules on the abaxial surface (Fig. 17.1A and B). Fruits infected with *Z. citri-griseum* show a typical rind blemish which significantly reduces the fruit quality for the fresh market (Fig. 17.1C). The pathogen incubation period is long and disease symptoms are only observed after 4–6 months (Mondal and Timmer, 2006). Microscopic observations show that *Z. citri-griseum* exhibits epiphytic growth on the leaf surface (Timmer et al., 2001). Secretion of nutrients or honeydew on the surface of the leaves also maximizes fungus survival (Whiteside, 1974).

17.1.3 Infection process

Z. citri-griseum belong to the *Mycosphaerellaceae* family that are characterized by the formation of pseudothecia that are in contact with the plant tissue. The typically ovoid and bitunicate asci carry ascospores, which also are responsible for the infection (Dewdney and Graham, 2016). Ascospores germinate and form epiphytic mycelia on the leaf surface that penetrate through stomata (natural openings of the abaxial leaf surface). Fungal growth in the leaf tissue is slow and symptoms develop several months later. Decomposing leaf litter in the orchard is the main source of airborne ascospores and primary inoculum (Mondal and Timmer, 2006).

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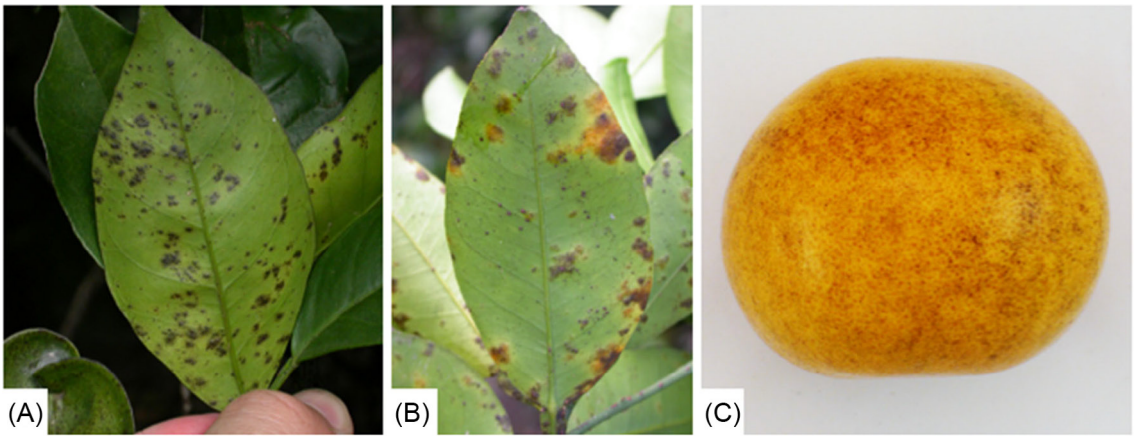


FIG. 17.1 Greasy spot disease symptoms in citrus caused by *Zasmidium citri-griseum.* Brown necrotic spots in the abaxial leaf surface in sweet orange (A) and in grapefruit (B). Rind blotch on grapefruit (C).

17.1.4 Pathogenicity and virulence

Zasmidium citri-griseum infects plants from the *Rutaceae* family such as citrus. Most citrus species appear to be susceptible, including the citrus relative *Poncirus.* Greasy spot development is favored in humid growing regions of citrus from the Caribbean to Florida in the US and in some cases are also present in semiarid areas. Greasy spot can affect most commercial citrus and its relatives. Fresh fruit market value of grapefruit (*Citrus paradisi*) is threatened by greasy spot rind blotch since grapefruit is highly susceptible. Besides grapefruit, other susceptible citrus species are sweet orange (*C. sinensis*), lemon (*C. limon*), and citrus hybrids such as tangelos are also considered susceptible based on field observations (Timmer et al., 2001).

17.1.5 Disease management

Copper is effective and still widely used to control greasy spot on leaves as it can directly kill ascospores and prevent infection. Copper can be used together with petroleum oils as additives to prevent penetration of the epiphytic mycelia. Petroleum oil sprays alone are not sufficient to control greasy spot rind blotch. Homogenous spray distribution of copper and oils on the leaf area is essential to protect the tree from future infections due to the epiphytic growth of *Z. citri-griseum* (Timmer et al., 2001). Copper applications can result in phytotoxicity on fruits when the temperature is >35°C and under dry weather conditions (Dewdney and Graham, 2016). Application of strobilurin or triazole fungicides is required to control this fungus in more susceptible citrus such as grapefruit, sweet orange, or in hot weather conditions. Timely spraying maximizes the efficacy of fungicide applications for greasy spot, and it is recommended to apply fungicide preventively prior to the ascospore penetration phase of the leaf surface (Dewdney, 2019).

17.2 Root rot, foot rot, brown rot of fruits, canopy blight, and damping-off diseases of citrus caused by *Phytophthora*

17.2.1 Introduction

Phytophthora species are among the most destructive oomycete plant pathogens known (Erwin and Ribeiro, 1996; Blair et al., 2008). They have a broad host range and are known as soilborne pathogens that mainly spread through the movement of infested soil, water, and infected plants (Goodwin, 1997). In Florida, *Phytophthora* species are the most economically important soilborne disease of citrus causing yield losses of up to 30% (Timmer et al., 2000). They infect all parts of the tree, including roots, stem, branches, twigs, leaves, and fruits. Major diseases caused by *Phytophthora* are root rot, foot rot or gummosis, fruit brown rot, canopy blight, and damping-off of seedlings (Cacciola et al., 2008) (Fig. 17.2). About 10 species of *Phytophthora* have been reported worldwide (Cacciola et al., 2008). *Phytophthora nicotianae, P. citrophthora,* and *P. palmivora* are the most predominant species in citrus (Graham and Menge, 1999; Graham and Timmer, 2006).

17.2.2 Disease symptoms

In general, *Phytophthora* species are favored by wet or flooded conditions (Graham and Feichtenberger, 2015). Foot rot (gummosis) is one of the most serious diseases caused by *Phytophthora* in citrus. Infection can occur at the ground

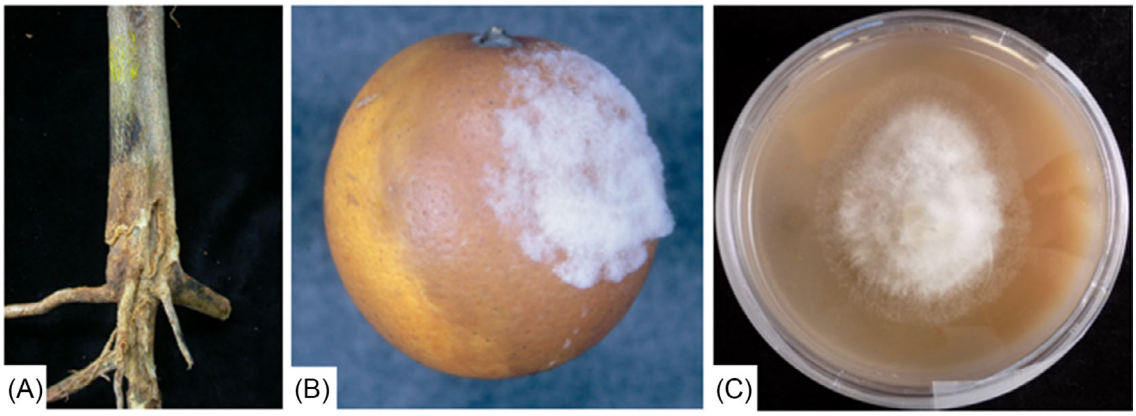


FIG. 17.2 Foot rot on Carrizo crown and roots (A) and brown rot on sweet orange (B) caused by *Phytophthora.* Strain of *P. nicotianae* cultured on V8 juice agar after 7 days showing typical white filamentous growth (C).

level or up the trunk of the tree (Fig. 17.2A). Symptoms of brown rot of fruit include light brown and leathery fruit rind (Fig. 17.2B). Brown rot is a common preharvest decay of citrus fruit that is favored by rainfall and results in preharvest fruit drop. Canopy blight symptoms include leaf chlorosis, defoliation, twig dieback, and weak growth of flushes and are commonly observed in citrus nurseries (Graham and Feichtenberger, 2015). Damping-off (seedling rot) is a disease of citrus in nurseries that affects the seedlings causing up to 80% mortality just before or after they emerge from the soil.

17.2.3 Infection process

Phytophthora exhibits filamentous growth similar to fungi (Fig. 17.2C), although they are genetically distant and more closely related to brown algae. Citrus tissues are most commonly infected through indirect contact with motile biflagellate zoospores that are released from sporangia, and less commonly from direct germination of sporangia (Klotz and De Wolfe, 1960; Graham and Timmer, 2006). Chlamydospores are resistant structures that allow the pathogen to survive in unfavorable conditions. Oospores are formed after sexual reproduction and act also as organs of preservation. Chlamydospore and oospore germination can occur in the presence of nutrient extracts from citrus (Savita et al., 2012).

17.2.4 Pathogenicity and virulence

Most commercial scion cultivars and rootstock can be infected (Feichtenberger et al., 1992). However, the use of tolerant scion-rootstock combinations is a useful tool toward overcoming root rot caused by *Phytophthora*. Rootstock responses to *Phytophthora* vary according to the species (Graham, 1995).

In Florida, *Phytophthora* species can interact with other plant pathogen microbes and pests (Graham and Feichtenberger, 2015). Diaprepes root weevil (*Diaprepes abbreviatus*) when in complex with *P. palmivora* can increase the severity of root rot damage and result in higher populations of the pathogen in the rhizosphere (Graham, 2000). *P. nicotianae* infecting roots also infected with *Candidatus* Liberibacter asiaticus (*C*Las, associated with HLB) has been reported to favor attraction of zoospores to roots and accelerate *Phytophthora* infection (Graham et al., 2011).

17.2.5 Disease management

It is essential to avoid *Phytophthora* in citrus nurseries by heat-treating seeds (50°C for 10 min) and planting them into sterilized, soilless media (Graham and Timmer, 2006). Adequate soil drainage in the orchards is essential because waterlogging induces buildup of *Phytophthora* populations in the soil, increasing the risk of infection (Graham and Menge, 1999). In established citrus orchards fungicides like copper have been highly effective for foot rot in citrus. Systemic fungicides like fosetyl-Al and metalaxyl/mefenoxam are used for foot root and root rot control in mature orchards. Metalaxyl/mefenoxam are also used in preplant applications to prevent damping-off (Graham and Timmer, 2006). Fungicide recommendations should be based on results of propagule counts in soil by conducting annual sampling. Fungicide applications are recommended to be continued for at least 1 year after initiation. Application methods include soil applications (soil drenching) and foliar applications (spraying the canopy). For control of foot rot, the microsprinkler irrigation system has been more effective. Brown rot of fruit can be managed with foliar applications of contact or systemic fungicides. Although chemical control is available, it can also be challenging as emerging fungicide resistant isolates have been reported (Timmer et al., 1998;

Thind et al., 2009). To avoid fungicide resistance development, it is important to first recognize other potential abiotic or biotic stress causing a decline of the trees before applying these fungicides (Graham and Dewdney, 2015). Chemical control cannot fully eradicate *Phytophthora* from citrus orchards. Another method of control is using *Phytophthora* resistant or tolerant citrus cultivars and rootstocks, for example, some *C. reticulata* (mandarin) and *P. trifoliata* and some of its hybrids with *Citrus,* show resistance or tolerance to *P. nicotianae*. Budding high on the rootstock and planting the trees on ridges avoid contact of the susceptible bark with rain or irrigation water carrying infective propagules. (Graham, 1990; Zhou et al., 1997; Graham and Menge, 1999; Guo and Deng, 2001; Mourã Filho et al., 2008; Boava et al., 2011; Yan et al., 2017; Dalio et al., 2018).

17.3 Melanose disease of citrus caused by *Diaporthe citri*

17.3.1 Introduction

Melanose is a disease of citrus forming corky pustules on twigs, young leaves, and fruits, which is caused by *Diaporthe citri* (= *Phomopsis citri*) (Fig. 17.3) (Fawcett, 1936). Melanose is only a serious problem in tropical and subtropical regions with fruit grown for the fresh market, in areas with high humidity and substantial rainfall during early fruit development (Gopal et al., 2014). Such places include, among others, Florida and eastern Australia. While the fungus may be found in many arid citrus-producing regions, it is usually not a problem. Leaf infections are not particularly important because, while leaf infection results in lesions, they are unlikely to significantly affect tree productivity or fruit internal quality. However, the disease results in superficial blemishes on the rind that can reduce the grade or render the fruit completely unsuitable for the fresh market. Melanose is not a serious problem when fruit is grown for processing (i.e., juice). After initial infection and pustule formation, melanose symptoms do not progress further.

17.3.2 Disease symptoms

The disease usually forms small (~1 mm diameter) reddish-brown to black pustules on fruit rind that may be randomly distributed on the surface ("flyspeck" melanose), or in patterns following locations where free water carried spores along the fruit surface ("tearstain" melanose) (Whiteside, 2000; Gopal et al., 2014). These symptoms have been sometimes confused with rust mite (*Phyllocoptruta oleivora*) damage, but rust mite blemishes are relatively smooth, whereas melanose pustules are raised and rough. Advances in multispectral imaging and computer programming have made distinguishing melanose from other rind blemishes possible using cameras on a grading line, although the technology is not yet commercially available (Zhang et al., 2018). Unfortunately, under certain conditions, copper sprays used to control the disease can themselves result in blemishes that look very similar to melanose and are sometimes called "star" or "stellate" melanose (Zhang et al., 2018). When *D. citri* infection is severe and early in fruit development (i.e., soon after petal fall), the pustules converge and form large patches of scar tissue that cracks as the tissue expands to form "sharkskin" or "mudcake" melanose symptoms (Fig. 17.3A). Lesions on leaves form small, black raised pustules that may be surrounded by a yellow halo early in development, but the chlorotic areas regreen over time (Fig. 17.3B). Leaves can become deformed if the shoot apex is severely infected when leaves are unfolding and, if leaf infection is severe enough, may cause general chlorosis and leaf abscission. Symptoms on twigs are similar to leaf symptoms, with severe infections resulting in shoot dieback.

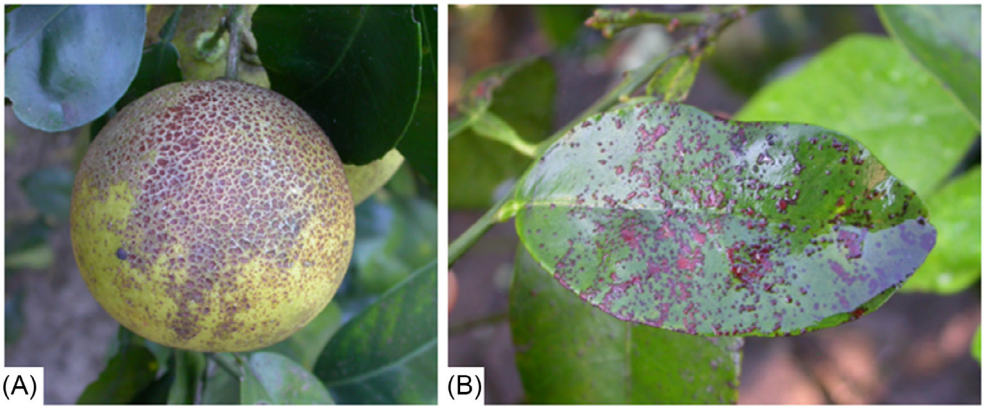


FIG. 17.3 Melanose disease symptoms on a fruit (A) and leaf (B) of grapefruit caused by *Diaporthe citri.*

17.3.3 Infection process

Diaporthe citri lives, grows, and reproduces primarily on deadwood, especially on twigs that died within the past 2–4 months. Asexually produced conidia are by far the most abundant spore produced and are primarily extruded under wet conditions and spread relatively short distances by rain and other water sources. Sexually formed ascospores can also be produced and are disseminated relatively long distances by wind but are not the main cause of infection. Spores landing on fruit or leaves directly penetrate epidermal cells via germ tubes secreting pectic enzymes, resulting in invasion and death of both epidermal and subepidermal cells up to about six cell layers deep, which become filled with a reddish-brown, gummy substance. Although the affected cells shrink by forming an initial slight depression, periderm forms beneath the dead cells about a week after infection, lifting the pustules and rupturing the cuticle to release the gum that then turns brown and hardens, imparting the characteristic raised and rough pustules.

The fungus does not develop after pustule formation on fruits and leaves and so does not produce spores, thus, fruits and leaves are not a source of inoculum for further infections. The amount of inoculum for new infections is related to the number of twigs within the canopy that died in recent months and are colonized by the fungus. Conditions that increase twig death, such as freezing injury, storm damage, insect pressure, mechanical pruning, etc. often lead to greater inoculum for disease development (Whiteside, 2000; Mondal et al., 2004).

17.3.4 Pathogenicity and virulence

While all citrus species are susceptible to melanose, grapefruit and lemon appear most affected, being more visible on the smooth, light-colored surface than on other varieties, and with larger pustules forming on grapefruit (Mondal et al., 2007; Dewdney, 2018). Leaves and fruits are susceptible to infection only during a limited period of development. Leaves are resistant after they become fully expanded. Fruits are resistant for about 3 months after petal fall, but the time period can vary based on the growing region (Gopal et al., 2014).

17.3.5 Disease management

Management of the disease is usually only needed when the fruit is intended for the fresh market and when the trees become old enough to have deadwood to support inoculum production. Managing trees to remove or avoid deadwood formation within the canopy is one way to minimize the disease. While pruning can be used, labor costs often preclude such practices, especially for large trees. Larger trees may benefit from topping if large amounts of deadwood are present in the canopy.

Fungicides are the main method used to control the disease, including copper- and strobilurin-containing fungicides (Dewdney, 2018). As a practical approach to control infection of deadwood or subsequent sporulation, fungicides would need to be applied continuously to have any effect. To protect fruit, copper fungicides are most commonly used because they are effective and more economical than other fungicides. However, copper can injure fruit if applied during hot weather, thus, other fungicides are often used during these periods (Albrigo et al., 2005).

The timing and frequency of fungicide application are critical considerations, especially in relation to fruit development and rain events that wash residues away. Fruit growth exposes new surfaces that are vulnerable to infection until the next fungicide application. In addition, localized heavy thunderstorms require more frequent fungicide reapplications. The first fungicide application must be applied before new fruit are exposed to rainy conditions. In Florida, this means they often do not occur until mid- to late-April, before the spring rainy season starts. After spraying starts, frequent fungicide applications at lower rates are usually the most effective. Repeated applications every 3 weeks are usually sufficient to control the disease, but a web-based tool is also available, which accounts for fruit growth and local weather events to calculate when the next copper application is needed (Dewdney et al., 2013). Fungicide applications can be ceased after the fruit has grown to a point when they are no longer susceptible (e.g., for grapefruit, about 7 cm diameter).

17.4 Citrus black spot disease caused by *Phyllosticta citricarpa*

17.4.1 Introduction

The citrus black spot disease (CBS) was first mentioned by Benson (1895) in New South Wales, Australia, consisting of fruit blemishes and premature fruit drop. The fungus was subsequently identified as *Phoma citricarpa* (McAlpine, 1899) and Koch's postulates were fulfilled by Lee (1920) and later by Kiely (1948), who also described the teleomorph of the fungus as *Guignardia citricarpa*. Based on current nomenclature, the accepted name of the CBS pathogen is *Phyllosticta citricarpa* (Glienke et al., 2011). Other *Phyllosticta* spp. have been associated with fruit and leaf spots in citrus, but in most cases, pathogenicity tests are lacking.

Sawada (1959) reported the presence of CBS in Taiwan. Lee (1920) indicated the presence of the disease also in mainland China, based on isolations from citrus fruit imported from China into the Philippines. In international databases, CBS was recorded as present in New Zealand, but further molecular studies concluded that the original isolate was indeed misidentified (Everett and Rees-George, 2006). In South Africa, CBS was reported by Doidge (1929) initially as a disease of minor importance in KwaZulu-Natal province. The disease is now widespread in many areas of sub-Saharan Africa. In Brazil, CBS was mentioned by Averna-Saccá (1940) but it was most likely a misdiagnosis, with a confirmed report four decades later. In Tucuman and Entre Rios, Argentina, CBS was first reported in the mid-1980s (Foguet et al., 1985; Garrán, 1996). In the Caribbean and North America, the disease was officially reported in Cuba in 2007 (Hidalgo-Góngora and Pérez-Vicente, 2010) and it was first observed in Florida in 2010 (Schubert et al., 2012). Citrus-growing areas in the Mediterranean basin were free of CBS, but *P. citricarpa* has been reported in Portugal, Malta, and Italy by Guarnaccia et al. (2017), however those findings were not confirmed by a later survey performed on the same sampling sites by the National Plant Protection Organizations of the three countries (EFSA Panel on Plant Health, 2018). Recently, outbreaks of CBS caused by *P. citricarpa* have been reported in India and Tunisia (Das et al., 2018; EPPO, 2019). In general, CBS epidemics are characterized by a long lag phase. The pathogen may be present for many years in an area before symptoms are first observed, and then decades until the disease reaches epidemic proportions, depending on climate and host susceptibility (Kotzé, 1981).

17.4.2 Disease symptoms

The CBS disease causes several types of rind blemishes on the fruit, reducing its marketability for fresh consumption. Lesions on fruit may be rather variable in appearance with symptoms generally being classified into different categories. Different categories of symptoms with different stages of development can be present on the same fruit. Moreover, some CBS symptoms can be easily confused with other diseases, abiotic disorders or pest damage. "Hard spot" is considered the most typical symptom of CBS, consisting of depressed lesions in the rind with a grayish center, dark margin, and yellow-to-green halo depending on fruit maturity (Fig. 17.4A). Hard spot lesions may remain 3–10 mm in diameter or coalesce into large lesions. "Freckle spot" consists of small spots about 1–3 mm in diameter, slightly depressed, mostly reddish and without any halo. Freckle spot lesions can also coalesce to form larger lesions. "False melanose" appears as small raised dark pustules, resembling those of the melanose disease caused by *D. citri*. Symptoms of "Virulent spot" extend into the albedo, forming sunken irregular lesions, variable in color, covering a great part of the fruit surface. Two additional miscellaneous symptoms have also been described, "Lacy Spot" consisting of superficial yellowish lesions, and "cracked spot" associated with the rust mite (IPPC, 2016).

Fruit symptoms usually develop on mature fruit at the time of color change, starting on the side most exposed to sunlight. Infected fruit may develop postharvest symptoms during transport or storage, particularly by increasing temperature (Brodrick and Rabie, 1970). Disease incidence is usually higher in old trees, with poor vigor and particularly in those subjected to drought stress (Kotzé, 1981). Disease incidences from 13% to 98% of fruit affected have been reported in untreated plots (Makowski et al., 2014). In addition to external fruit blemishes, CBS can also induce premature fruit drop and yield losses over 60% have been reported in Brazil (Silva-Junior et al., 2016). On leaves, CBS is usually asymptomatic and *P. citricarpa* develops as quiescent infections. In highly susceptible citrus species, such as lemon (*C. limon*) or stressed



FIG. 17.4 Citrus black spot disease symptoms on fruit (A) and leaf (B) of sweet orange caused by *Phyllosticta citricarpa.* Strain of *P. citricarpa* grown on potato dextrose agar (PDA) (C).

trees, foliar symptoms of CBS may appear as circular light brown spots up to 3 mm in diameter, with dark margins and a yellow halo (Fig. 17.4B). Similar symptoms can also be present on twigs, but 0.5–2 mm in diameter (IPPC, 2016).

17.4.3 Infection process

The species *P. citricarpa* is heterothallic and complementary mating types are needed for sexual reproduction (Tran et al., 2017). Sexual reproduction occurs in the leaf litter, where pseudothecia, asci, and ascospores are produced. Alternate wetting and drying and rising temperatures favor pseudothecia maturation and ascospore production. Conversely, an excessively moist environment induces more rapid leaf litter decay and the formation of pseudothecia is negatively affected (Lee and Huang, 1973). Studies conducted in South Africa indicated that once mature, the release of ascospores is mainly driven by rain (Fourie et al., 2013). Ascospores are airborne and can disseminate relative long distances, though quantitative data on their dispersal range are not available. In Taiwan, airborne ascospores were trapped mainly from May to October, in Brazil from November to March, and in South Africa from October to February (Lee and Huang, 1973; Reis et al., 2006; Fourie et al., 2013). Nevertheless, the spore traps used by these studies could not differentiate *P. citricarpa* from the coexisting endophyte *P. capitalensis*. Therefore, results should be interpreted with caution.

Asexual reproduction of *P. citricarpa* occurs in fruit lesions, twigs, and leaf litter. Conidia are produced in pycnidia and are dispersed by rain splash. Laboratory studies with simulated wind-driven rain indicated that *P. citricarpa* conidia can disseminate at least 8 m and to heights of 75 cm (Perryman et al., 2014). Based on studies conducted in South Africa, ascospores were generally considered the main, if not the only, source of inoculum for CBS epidemics. Conidia were historically deemed to have epidemiologically a minor role, only for fruit-to-fruit infections when mature fruit with lesions remain on the canopy after fruit set. However, studies conducted in Brazil indicated that conidia produced on twigs may be another important source of inoculum (Spósito et al., 2008; Silva-Junior et al., 2016). Epidemics of CBS in Florida, have recently been associated with a clonal population of *P. citricarpa*, with only one mating type, further supporting the importance of asexual reproduction and conidia under certain conditions (Wang et al., 2016; Hendricks et al., 2017).

Ascospores and conidia of *P. citricarpa* germinate and infect under adequate conditions of temperature and moisture. Nevertheless, the specific temperatures and wetness durations required for infection have not been determined. Citrus leaves remain susceptible to infection for up to 8–10 months after their emergence (Truter, 2010). In South Africa, fruit are considered susceptible to infection during 4 months after fruit set (Kotzé, 1981) but longer susceptibility periods up to 6–7 months were reported in Ghana and Brazil (Brentu et al., 2012; Lanza et al., 2018). The pathogen penetrates into the host by forming appressoria and establishes latent infections between the cuticle and epidermis (McOnie, 1967). The latent, asymptomatic period lasts for several months and CBS symptoms are visible only when fruit is fully developed, and ripening starts (i.e., color break). Symptom expression is enhanced by rising temperatures, high light intensity, drought stress, and tree age (Kotzé, 1981).

17.4.4 Pathogenicity and virulence

All commercial citrus species and cultivars are considered to be susceptible to CBS, except for sour orange (*C. aurantium*) and Tahiti lime (*C. latifolia*) (Kotzé, 1981; Baldassari et al., 2008). Apparently, *P. citricarpa* can colonize tissues of these two citrus species but without inducing CBS symptoms (Baldassari et al., 2008; Wickert et al., 2009). Lemon is considered the most susceptible to CBS and in some areas the first disease outbreaks were spotted on this citrus species. Conversely, other regions reported the first disease foci in sweet orange orchards. Late-maturing cultivars of sweet orange, like those of the Valencia group, were considered more susceptible to CBS and indeed they show higher disease severity. However, this may be due to the longer period the fruit of these late-maturing cultivars hang on the canopy, interacting with the environmental and physiological factors leading to symptom expression.

17.4.5 Disease management

Quarantines limiting or prohibiting the movement of citrus material were established as an exclusion measure to avoid the entry of *P. citricarpa* into new areas (Martínez-Minaya et al., 2015). For eradication, Whiteside (1967) proposed drastic pruning, leaving only the framework limbs, together with leaf litter elimination as an alternative to tree removal. However, despite the relatively slow epidemic progression of CBS, successful eradication was not accomplished anywhere. As Kotzé (1981) pointed out, CBS advances slowly but rarely retreats.

Using healthy plant propagation material and cleaning plant debris from incoming vehicles are essential to avoid the introduction of CBS into the orchard. Once established in the orchard, cultural practices are recommended to manage CBS.

Maintaining optimal tree condition by proper fertilization and avoiding drought stress may reduce CBS severity. Irrigation can be also used to synchronize flowering and avoid secondary infections from mature to young fruit. Early harvest, before symptom expression and subsequent fruit drop, may reduce yield losses. Nevertheless, latent infections on fruit can still develop symptoms during transport and storage. Various cultural practices were evaluated to reduce ascospore inoculum in the orchard by leaf litter removal, enhancing leaf decomposition or establishing cover crops (Bellotte et al., 2009, 2013; Van Bruggen et al., 2017). However, poor disease control was obtained in Brazil when implementing some of these practices at large scale (Spósito et al., 2011), probably owing to the concurrence of additional inoculum sources like conidia produced on twig lesions.

Fungicide treatments are generally needed for CBS management in the field. Different combinations of copper compounds, dithiocarbamates, strobilurins, and benzimidazoles have been used (Makowski et al., 2014). In South Africa, the disease was effectively controlled by a single spray of benomyl when applied as a curative treatment after the infection period (Kotzé, 1981). Over time, *P. citricarpa* developed resistance to benomyl (Schutte et al., 2003) and nowadays CBS control is based on preventive spray programs to protect the fruit from infections. The number of sprays per growing season may vary from 2 to 6 depending on the prevailing climate conditions, inoculum availability, cultivar, and fruit susceptibility period (EFSA, 2014b). The destination of the fruit also determines the spray programs, because fruit quality standards are much more stringent for fresh consumption than for industrial processing. Postharvest treatments cannot effectively control latent infections in fruit (Yan et al., 2016).

17.5 Brown spot, leaf spot and black rot diseases of citrus caused by *Alternaria*

17.5.1 Introduction

Alternaria species are generally known as necrotrophyc fungi, and several are pathogenic to citrus and cause significant yield losses. Alternaria diseases of citrus include Alternaria brown spot of tangerine (*C. reticulata*) and their hybrids, Alternaria leaf spot of rough lemon (*C. jambhiri*) and Rangpur lime (*C. limonia*), Alternaria black rot of citrus fruits, and "mancha foliar" disease, caused by *A. limicola*, that occurs only on Mexican lime in Mexico (Palm and Civerolo, 1994). *Alternaria* species causing these four citrus diseases are difficult to distinguish morphologically, thus, pathogenicity tests, toxin assays, and phylogenetic analysis based on sequence data from several genes are usually required to distinguish them.

Symptoms of Alternaria brown spot of tangerine were first reported on "Emperor" mandarin in Australia in 1903 (Cobb, 1903). It appeared in Florida in 1974 and subsequently was identified in other citrus production areas including Israel (Solel, 1991), Cuba (Herrera-Isla, 1992), Turkey (Canihos et al., 1997), South Africa, Colombia (Timmer et al., 2000), Spain (Vicent et al., 2000), Italy (Bella et al., 2001), Brazil, and Argentina (Peres et al., 2003). Recently, this disease was also reported in Southeast Asia and caused significant losses on several important cultivars in China (Wang et al., 2010; Huang et al., 2015a). Alternaria leaf spot of rough lemon was first reported in South Africa in 1929 (Doidge, 1929). This disease affects only rough lemon and Rangpur lime, two important rootstocks in some citrus-growing areas. Thus, Alternaria leaf spot of rough lemon is only significant in nurseries and seed production blocks. Alternaria black rot of fruit was first described as early as 1900s in California (Pierce, 1902; Cobb, 1903). It is a significant postharvest disease for all species of citrus under the appropriate conditions but occurs most commonly on Navel oranges (*C. sinensis*) in the field prior to harvest. Alternaria black rot occurs in all citrus-producing regions but is more prevalent in production areas with Mediterranean climates, characterized by cool and moist winters and hot and dry summers, than in humid areas.

17.5.2 Disease symptoms

Host specificity and pathogenicity of Alternaria brown spot and Alternaria leaf spot pathogens are associated with the production of host-selective toxins (Ohtani et al., 2009). Alternaria brown spot affects young leaves, twigs, and fruits, producing brown-to-black lesions surrounded by a yellow halo (Kohmoto et al., 1979; Meena et al., 2017a) (Fig. 17.5A and B). Leaf lesions are generally circular, extending along the veins as the toxin spreads in vascular tissues, which gives the lesions an eye-spot appearance with a tail (Timmer et al., 2004). On young leaves of very susceptible cultivars, lesions can appear as early as 24 h after infection. Lesions enlarge as leaves mature and can vary in size from 1 to 10 mm and will extend to whole leaves if the conditions, such as moisture, temperature, and susceptibility of cultivars, are appropriate (Fig. 17.5A). The yellow halo is usually more evident on full-size but immature leaves. When this disease is severe, the affected leaves often abscise. Infected young shoots also show brown-to-black sunken lesions, the entire young shoots will dieback, especially if the leaves have fallen (Timmer et al., 2000).

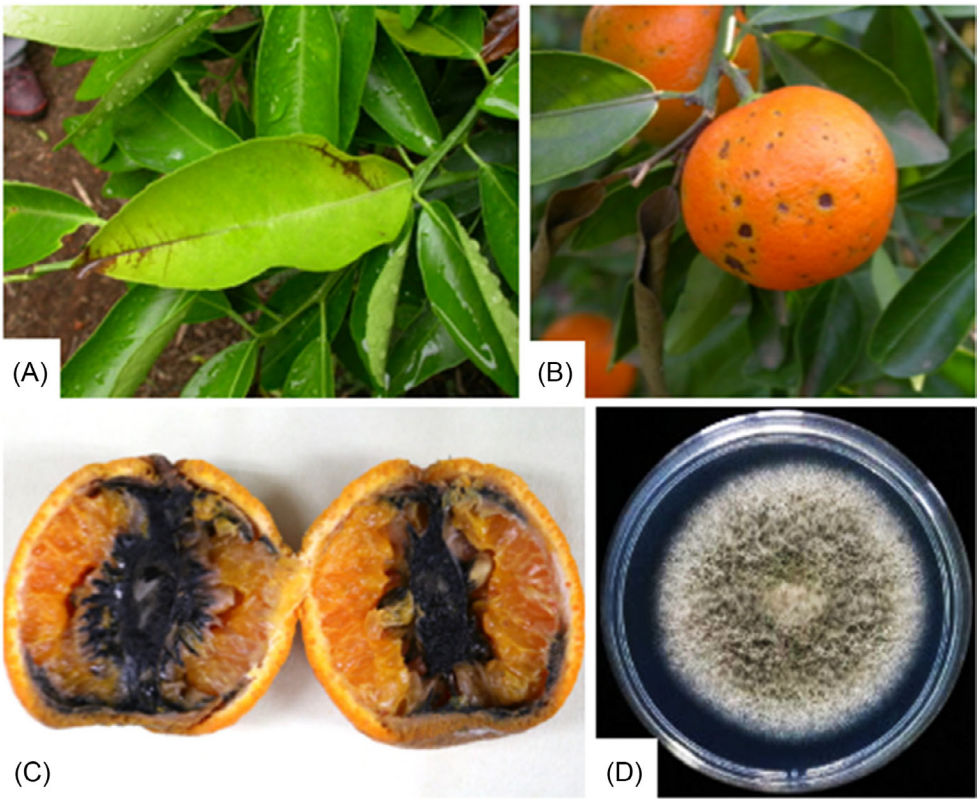


FIG. 17.5 Symptoms of Alternaria brown spot in "Fortune" mandarin leaves (A) and fruit (B). Symptoms of Alternaria black rot in sweet orange fruit (C). Strain of *Alternaria alternata* tangerine pathotype on the PDA (D).

In the case of Alternaria brown spot, young fruits can be infected shortly after petal fall; the lesions are brown to black, sunken without a yellow halo surrounding them. When the fruits grow, larger than to 2–3 cm in diameter, the lesions become sunken, turn to gray in the center and are surrounded by a yellow halo (Fig. 17.5B). The infected fruits drop off rapidly, reducing yield. For the very susceptible cultivars, such as 'Hongju' (also known in the western countries as "Dancy"), the fruit is susceptible to Alternaria brown spot during the whole growing season but, in general, fruit is susceptible for 4 months after petal fall. Even when the fruit is no longer susceptible, some fruits may fall as the result of earlier infections, especially if lesions are near the fruit peduncle. Alternaria leaf spot of rough lemon affects only rough lemon and Rangpur lime. Symptoms on leaves are very similar to those produced by Alternaria brown spot and do not appear for about 3 days after inoculation. Symptoms on fruit are merely small brown specks and are quite reduced relative to the tangerine pathotype (Timmer et al., 2000). Alternaria black rot affects the central columella of the fruit (Fig. 17.5C) and can affect all species of citrus (Brown and McCornack, 1972). External symptoms are often not apparent and, if present, appears as a small brown-to-gray spot on the stylar end of the fruit (Brown and Eckert, 2000). Affected fruit are more brightly colored than normal fruit due to ethylene generated in response to infection.

17.5.3 Infection process

The pathogen of Alternaria brown spot overwinters mainly on diseased fruits, twigs, or mature leaves. In the spring of the following year, the spores produced on the diseased leaves spread through the airflow and then are deposited on susceptible tissues. Rainfall and dew favor the infection of this pathogen. When environmental conditions are conducive, spores germinate and then produce ACT-toxin to kill the host cells for nutrients. The ACT-toxin quickly spreads through the vascular system, accelerates cell necrosis and enlargement of the lesion, and in severe cases, eventually leads to leaf and fruit drop. Spore production on the diseased tissue begins about 10 days after the lesions are well developed and continues for about 50 days (Dewdney, 2016).

The pathogen of Alternaria black rot overwinters mainly as spores or mycelium on diseased fruits or other substrates. Under suitable temperature and humidity conditions, this fungus produces spores that are spread by airflow on to the receptacle or young fruit, and then those spores remain dormant until the anaphase of fruit growth. During the anaphase of

fruit growth or postharvest storage, the hyphae invade from the natural crack, wound or pedicle, causing decay. This disease is greatly affected by environmental factors and the degree of fruit maturity at harvest. The moist conditions facilitate the growth and sporulation of the pathogen on dead citrus tissue. The optimum temperature for growth of the pathogen is around 25°C (Timmer et al., 2000). It is not conducive to mycelial growth when the temperature is lowered to 12–14°C. Poor irrigation and drainage, poor cultivation management, citrus orchards with weak trees, fruits suffering from sunburn, insect caused wounds, or mechanical damage promote the occurrence of Alternaria black rot.

17.5.4 Pathogenicity and virulence

Alternaria brown spot and Alternaria leaf spot pathogens are under taxonomic reassessment (Woudenberg et al., 2015). Alternaria brown spot was described to be caused by *A. alternata* tangerine pathotype on some cultivars of tangerine (*C. reticulata*) and some hybrids of tangerine × grapefruit (*C. reticulata* × *C. paradisi*) or tangerine × sweet orange (*C. reticulata* × *C. sinensis*). This pathogen produces the host-selective ACT-toxin (Kohmoto et al., 1993), which can cause lesions or kill the whole leaf even when it does not colonize the leaf tissue. Young leaves are very susceptible to this pathogen whereas old leaves are resistant. Alternaria leaf spot of rough lemon and Rangpur lime was described to be caused by *A. alternata* rough lemon pathotype (Timmer et al., 2003). This pathogen produces the host-selective ACR-toxin or ACRL-toxin (Gardner et al., 1985; Nakatsuka et al., 1986; Meena et al., 2017b) that kills host cells for nutrients and reproduction on diseased tissue. Alternaria black rot of fruit is caused by *A. alternata* or some other species (Brown and Eckert, 2000) on all citrus cultivars such as lemon, lime, sweet orange, mandarin, and grapefruit (Gardner et al., 1985; Katoh et al., 2006). Natural cracks or wounds are required for penetration of the fungus (Timmer et al., 2003). Some studies have shown that the occurrence of Alternaria black rot of fruit is related to the activity of endopolygalacturonase (Isshiki et al., 2001).

17.5.5 Disease management

Fungicides are the primary means for the control of Alternaria brown spot. Copper compounds are the most economical fungicide to control Alternaria brown spot on citrus (Vicent et al., 2007). Spraying at spring flush (2–3 cm) with a second application 7–14 days later using copper compounds protect young leaves from infection and inhibits sporulation. Further sprays are aimed mainly to protect fruit from infection. The strobilurin fungicides can also be used but with a proper strategy to avoid fungicide resistance as a substitute when the weather is dry and hot to avoid copper damage to fruit (Timmer et al., 2003). Using disease-free planting material, pruning affected tissues, leaf litter management, and improving cultivation measures can be used to reduce disease severity. It is important for susceptible cultivars to be planted rather with a wide-spacing on higher ground with good air ventilation and light penetration so that the canopy may dry more rapidly. Avoiding excessive watering and nitrogen application, and increasing organic fertilizer, phosphorus and potassium applications can reduce disease severity in susceptible citrus cultivars.

Cultural practices such as trimming to improve the ventilation and light penetration, removal of diseased fruits, twigs, and leaves to reduce inoculum sources and avoiding overfertilizing and watering are effective in controlling Alternaria leaf spot (Timmer et al., 2000). For the control of Alternaria black rot, preventing fruit stress, trimming and pruning trees to improve air circulation and light permeability of the orchard, cutting off dead, weak, diseased, or dense branches to prevent fruit abrasions and stylar damage, as well as harvesting fruits at optimum maturity can reduce disease incidence (Timmer et al., 2000, 2003).

17.6 Postbloom fruit drop disease caused by *Colletotrichum*

17.6.1 Introduction

Postbloom fruit drop (PFD) disease of citrus, caused by *Colletotrichum acutatum,* is an economically important fungal disease of citrus occurring in the humid tropical areas of the Americas and sporadically in the humid subtropics (Timmer et al., 1994). In Florida, yield losses due to PFD have been observed in some years when rainfall was high during spring flowering. The pathogen infects all citrus cultivars and causes peach-to-orange-brown necrotic lesions on petals with salmon pink acervuli, necrosis of stigma, and premature fruit drop (Denham and Waller, 1981) and retention of calices called “buttons” (Fig. 17.6). Although PFD-like disease symptoms were observed in various areas of Central and South America in the 1950s and 1960s (Timmer et al., 1994), it was not reported until in the late 1970s from Belize (Fagan, 1979). The disease is now widespread in all humid citrus areas from Florida (USA) in the north to Uruguay in the south. It is endemic in many humid tropical areas such as southern Mexico, Belize, Costa Rica, and many islands in the Caribbean (Timmer and Peres, 2015).

In other areas such as Brazil, it is also a problem, when flowering coincides with a rainy period in which the crop losses can exceed 80% (De Goes et al., 2008). Thus far, the disease appears to be confined in the Americas because it has not been reported from other parts of the world.

Morphological characteristics such as colony morphology, conidial shape, the presence or absence of setae and sclerotia, and appressorium shape and size have been traditionally used for differentiation of species in the genus *Colletotrichum* (Sutton, 1992). Most of the fungi currently included in *C. acutatum* were formerly assigned to *C. gloeosporioides* with the teleomorph being *Glomerella cingulata* (Baxter et al., 1983; Van Der Aa et al., 1990; Sutton, 1992). In fact, the citrus PFD pathogen was also originally identified as *C. gloeosporioides* (Agostini et al., 1992; Timmer et al., 1994), but was later found to be *C. acutatum* (Brown et al., 1996). Nevertheless, the species *C. gloeosporioides* has also been reported recently as a causal agent of PFD (Lima et al. 2011; McGovern et al. 2012). Recent comprehensive studies of *Colletotrichum* species-complexes using multilocus analyses (Damm et al., 2012; Weir et al., 2012), and a new locus like ApMat (Sharma et al., 2015.) have substantially contributed to the better understanding of *Colletotrichum* diversity on citrus. At least 15 *Colletotrichum* species have been reported associated with citrus (Damm et al., 2012; Weir et al., 2012; Sharma et al., 2015). Known *Colletotrichum* diversity on citrus is based on studies with spatially limited sampling, thus, a geographically comprehensive study with proper pathogenic characterization is still lacking (Peres et al., 2008; Hou et al., 2010).

Colonies of *C. acutatum* are usually white initially and later become covered with pink-to-orange conidial aggregates (Zulfiqar et al., 1996). Conidia are primarily produced in acervuli; however, *C. acutatum* is also capable of forming secondary conidia on the surface of living leaves (MacKenzie et al., 2010), and it has recently been reported that secondary conidia can survive for up to 3 months on different weed species commonly found in citrus orchards in Brazil (Frare et al., 2016). Conidia of *C. acutatum* are usually ellipsoid and fusiform at one end and are different than those of *C. gloeosporioides*, which typically are rounded at both ends (Adaskaveg and Förster, 2000).

17.6.2 Disease symptoms

The disease in citrus is usually first observed as a peach-to-orange-colored necrotic lesions on open flowers (Fig. 17.6). Petals on open flowers are the most susceptible to infection, however, unopened flower buds may be infected (Timmer and Peres, 2015). The internal organs of the flower, as well as the pollen, can also be infected (Lin et al., 2001; Marques et al., 2013). In severe cases, whole flower clusters may be attacked causing blight of entire blossoms with shriveled necrotic petals stick firmly to inflorescences. After petal fall, the calyces and floral disks which normally abscise if no fruit is set, remain attached to the twig (Fig. 17.6A). These calyces, commonly called "buttons," persist for the life of the twig. Thus, signs of the disease are usually present year-round even though the infection occurs only during bloom. The buttons are characteristic of the disease and are rarely associated with any other disorder. Leaves surrounding infected flowers are often distorted with twisted laminae and enlarged veins (Timmer and Peres, 2015).

17.6.3 Infection process

The fungus is highly dependent on rainfall, especially frequent rain events when flowers are present in the orchard. Infection usually happens within 24 h of a favorable weather event, and symptoms occur in approximately 4–5 days (Agostini et al., 1992; Feichtenberger and Spósito, 2000b), In petals, penetration occurs intra- and intercellularly and



FIG. 17.6 Disease symptoms of postbloom fruit drop on Valencia sweet orange in the calyx (button) (A) and typical orange lesions on flower buds (B). Strain of *Colletotrichum acutatum* isolated from citrus flowers grown on potato dextrose agar (PDA) (C).

through the stomata (Marques et al., 2013). Copious conidia formed in acervuli on the surface of infected flowers are readily dispersed to healthy flowers with water/rain splash (Timmer et al., 1994). During the season, the disease continues to spread in an orchard as long as flowers are available particularly if trees are under continuous stress (e.g., HLB) and environmental conditions are favorable. Conidia deposited on vegetative surfaces, including weed hosts on the orchard floor, form appressoria, and latent infections that serve as survival structures during periods when flowers are absent. These appressoria will germinate to produce secondary conidia when exposed to petal leachates under moist conditions (Agostini et al., 1992; Timmer et al., 1994; Zulfiqar et al., 1996; MacKenzie et al., 2010; Frare et al., 2016). Thus, the first petals produced in the spring probably stimulate the germination of appressoria and the formation of conidia to initiate a new infection cycle. Moreover, at least in Brazil, weeds may also serve as an inoculum source for infection of citrus flowers during periods of bloom (Frare et al., 2016). This might indeed explain the sudden outbreaks of PFD in an orchard during years that had favorable climatic conditions for the disease, even in areas where the disease had not been previously reported (Timmer et al., 1994).

The lifestyles of *Colletotrichum* species can be broadly categorized as necrotrophic, hemibiotrophic, latent or quiescent and endophytic; of which hemibiotrophic is the most common. The differences in lifestyle depend on the *Colletotrichum* species, the host species, the physiological maturity of the host and environmental conditions. In citrus, *C. acutatum* infection strategies lead to a unique lifestyle; as a necrotroph on flowers and as a biotroph on leaves, thus, reproduces primarily on flower petals and does not readily colonize and form acervuli on other tissues (Agostini et al., 1992; Timmer et al., 1994; Zulfiqar et al., 1996; Peres et al., 2005).

While windblown rain is the main means of disease spread, human activity contributes to long-distance spread through equipment movement, especially if such movement occurs while the canopy is wet. Bees, fruit flies, and other flower-visiting insects are known to carry conidia of *Colletotrichum* (Pena and Duncan, 1989). Honeybees (*Apis mellifera*), as pollinating insects, are usually abundant in citrus orchards during the bloom period. In fact, recent studies have shown that the conidia of *Colletotrichum* were attached to parts of honeybee bodies and were dispersed among citrus plants in the greenhouse by honeybees (Gasparoto et al., 2017). Additionally, pollen grains can also be infected by the pathogen (Marques et al., 2013). Thus, it is also possible that bees carry conidia of *C. acutatum* directly on their legs and that the pathogen may survive in beehives. Nevertheless, this assumptions and roles of both infected pollen and honeybee or other pollinators in the epidemiology of PFD under field conditions should be determined with further studies.

17.6.4 Pathogenicity and virulence

Several *Colletotrichum* species have been shown to exhibit substantial pathogenic variation (Cannon et al., 2012). Differences in pathogenicity of strains from different hosts have been reported in several studies. Some fruit diseases caused by the *C. acutatum*-complex have been shown to be caused by distinct phylogenetic lineages (Peres et al., 2008), and strawberry fruit rot, in particular, was rarely found to be caused by isolates from diverse hosts (MacKenzie et al., 2009). The comparison of isolates of *C. acutatum* from sweet orange, Key lime, strawberry, blueberry, and leatherleaf fern have shown little evidence of cross pathogenicity. Furthermore, sequences of some genes in isolates from the same host were found similar or identical to each other but distinct from those isolates from other hosts (MacKenzie et al., 2010).

The mechanisms involved in young fruit abscission are suggested to be associated with a series of physiological modifications caused by an imbalance of plant hormones and/or growth regulators (Lahey et al., 2004). Until recently, PFD has been attributed exclusively to *C. acutatum*, whereas *C. gloeosporioides* was thought to only cause postharvest anthracnose in citrus fruits and can be found epiphytically on leaves and flowers (Brown et al., 1996; Peres et al., 2005). It was speculated that epiphytic *C. gloeosporioides* isolates might have shifted into being pathogenic in Brazil and those isolates of *C. gloeosporioides* that cause PFD are apparently less aggressive than those of *C. acutatum* (Lima et al., 2011). Consistent with this hypothesis, disease incidence, assessed by blossom blight or by persistent calyces, was found to be lower when plants were inoculated with *C. gloeosporioides* than with *C. acutatum* (Lima et al., 2011).

17.6.5 Disease management

The management of PFD requires an integrated disease strategy consisting of adequate cultural practices and timely application of effective fungicides or biocontrol agents. Effective cultural practices to prevent PFD outbreaks include avoiding stress conditions in citrus trees due to abiotic (i.e., irrigation, fertigation, or chemical) and biotic (i.e., disease) factors. This, in turn, will promote more compact bloom periods and will delay inoculum buildup and prevent serious epidemics in the orchard. As a matter of fact, PFD in Florida in recent years has reemerged with severe outbreaks after many years

of minimal incidences in the state and is probably due to increased tree decline and off-season flowering caused by HLB, which is now endemic in the state. In some areas, overhead irrigation was used, which greatly extended the wetting periods and splash dispersed inoculum within trees and to nearby trees. Switching to micro sprinkler or drip irrigation has helped reduce PFD severity (Timmer and Peres, 2015).

Adequate control of PFD is highly dependent on timely applications of effective fungicide sprays in the bloom period. Timing is highly specific to the orchard situation, including overall flowering stage and local weather conditions and can be complicated because of inconsistent and off-season bloom that usually occur in HLB endemic areas (e.g., Florida and Brazil).

There are a number of fungicides shown to be effective on *Colletotrichum* species in citrus. Among those, benomyl and captafol were the most effective fungicides for PFD control in the past (Fagan, 1984; Timmer and Zitko, 1992). Captan, maneb, and other contact fungicides also provided some control of the disease. Carbendazim was also effective against PFD (De Goes et al., 2008; Silva-Junior et al., 2014) and would be a substitute for benomyl, which has been removed from the market. However, the use of the benzimidazole fungicides, like thiabendazole, carbendazim, and thiophanate-methyl, has also been recently restricted (Timmer and Peres, 2015). Folpet, a member of the same fungicide group as captan, has also proven quite effective for control of PFD in Brazil (Feichtenberger and Spósito, 2000a; De Goes et al., 2008). In Florida, Timmer and Zitko (1998) found that strobilurins, such as trifloxystrobin and azoxystrobin, provided some control of the disease, but were not as effective as benomyl. Silva-Junior et al. (2014) found that the mixture of trifloxystrobin + tebuconazole was the most effective in studies in Brazil. Although many products on the market provide some degree of control of PFD, growers have been left with no highly effective materials. Thus, control of PFD has been made even more challenging (Timmer and Peres, 2015).

17.7 Citrus scab diseases caused by *Elsinoë*

17.7.1 Introduction

The causal agents of scab diseases of citrus are *Elsinoë fawcettii* (= *Sphaceloma fawcettii*) and *E. australis* (= *S. australis*). Scab diseases of citrus are distributed in many citrus-producing countries worldwide in humid zones except Mediterranean regions. These pathogens affect the fruits, leaves, and twigs of susceptible species of citrus, including lemons, grapefruits, many tangerines and their hybrids causing external blemishes and reducing the acceptability of fruits for the fresh market (Chung, 2011). Two scab diseases are currently recognized on citrus: citrus scab, caused by *E. fawcettii*, and sweet orange scab, caused by *E. australis*. *Elsinoë* isolates were differentiated into at least six pathotypes by pathogenicity and genetic analysis: Florida broad host range (FBHR), Florida narrow host range (FNHR), Tryon's, "Lemon," "Jingeul," and *E. australis* natsudaidai pathotype (Timmer et al., 1996; Hyun et al., 2009). The teleomorphs of both species of *Elsinoë* were only found in Brazil. The conidia have two types: hyaline conidia and spindle-shaped colored conidia (Timmer et al., 2000). Colony morphology of *E. australis* and *E. fawcettii* cultured on PDA has two types, mucoid and tomentose (Fig. 17.7A–C). Although the same isolate exhibits two colony morphologies, it is not certain whether the colony morphology type is determined under specific conditions. However, most of the *Elsinoë* strains isolated from lesions initially have a tomentose type, and when they are subcultured, they appear to be mainly mucoid type.

17.7.2 Disease symptoms

The scab pathogens induce typical symptoms on adaxial as well as abaxial surfaces of the leaves (Fig. 17.7D). These symptoms begin to appear 3 days after pathogen invasion, with tissues around the infection sites becoming yellowish and a small spot appears in the center. Initially, scab pustules are slightly raised and pink-to-light (Fig. 17.7E) brown and, as the pustules develop, they become warty and cracked and turn yellowish-brown and eventually dark gray (Fig. 17.7F) (Whiteside, 1975; Chung, 2011). Elevation and size of the pustules vary according to disease sensitivity, tissue age at the time of infection, and environmental conditions. Elevated protuberances form on the side of the leaf that has been invaded, and a corresponding indentation forms on the opposite side. In severe cases, the young leaves or young fruits become deformed and even drop from the tree. Symptoms vary depending on the developmental stage of the leaf or fruit (Timmer et al., 2004). Protuberance-type symptoms are induced in early infections and gradually become flatter as the tissue grows larger. However, as the fruit grows larger, the protruding-type or the scab-type lesion turns into a flatter or scurfy-type lesion. Scab symptoms, especially the flatter and scurfy-type lesions that occur on fruits, are easily confused with gray mold (*Botrytis cinerea*). In addition, in resistant cultivars or almost expanded leaves and when environmental conditions are not suitable, lesions may appear as black spots.

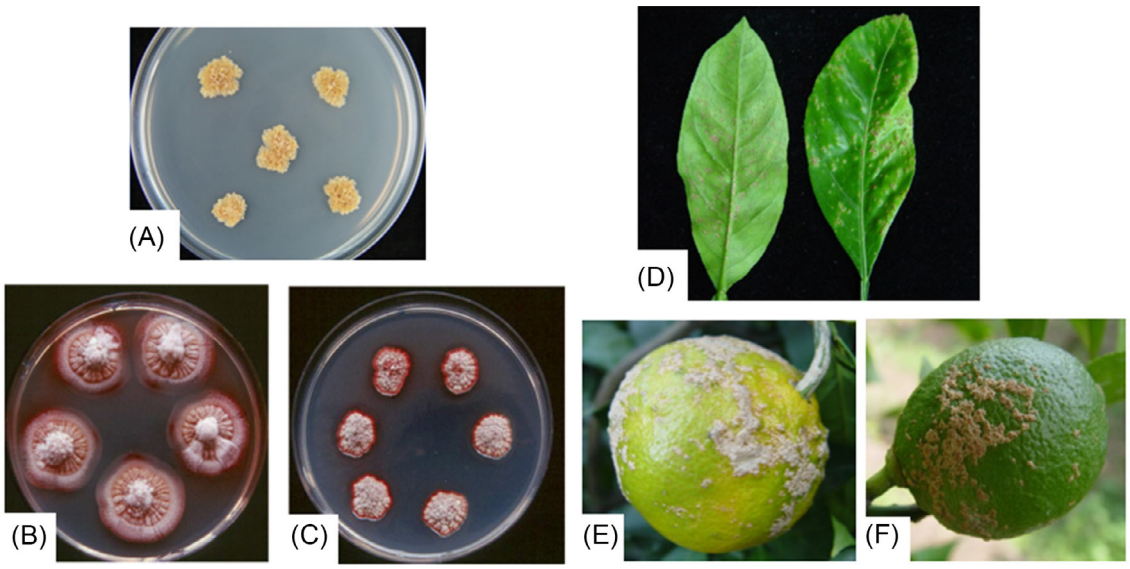


FIG. 17.7 Colony morphology of *Elsinoë* showing mucoid (A) and tomentose type (B and C) grown for 20–30 days on plates with potato dextrose agar (PDA). Symptoms of scab showing elevated protuberances in leaves (D) and scab pustules in fruits from *light* (E) to *dark gray* (F).

17.7.3 Infection process

Conidia of the citrus scab pathogens infect on adaxial as well as abaxial surfaces of the leaf. Conidia extend their germ tubes from 1-day postinoculation with globose appressorium-like structures at their tip. Cuticle and mesophyll cells including epidermis in the infected dark region degrade 1–2 day-postinoculation, resulting in cellular necrosis. The adjacent cells of the necrotic region enlarge and are gradually covered leaving a groove-shaped necrotic center and appear as a first visible symptom after 3 days (Paudyal et al., 2017). Hyphae extend through the necrotic epidermis and colonize intra- and intercellularly in mesophyll tissue. Also, lamellated cell-walls are observed as a boundary between infected and noninfected regions in the lesion after typical symptoms were formed. Cross sections through the lesion reveal hyphal colonization in epidermis and mesophyll tissues. In response to the pathogen colonization, host cell walls are lignified, inner cells are rapidly compartmentalized, and a semicircular boundary is formed that separates the infected region from the noninfected region, and finally prevents the intercellular pathogen spread (Paudyal and Hyun, 2015). The pathogen overwinters in lesions on twigs, leaves and remaining fruit, and when the lesions are wet at 15–20°C for 2 days, it initiates production of conidia for infection. The rate of disease progression is fastest at around 25°C and the first symptom appears within 3 days after infection. The disease does not occur above 35°C. Overwintered lesions on leaves are very important as a first inoculum source in the field. Once conidia are formed, pathogens can invade even if the plant surface is wet for about 3 h at the optimum temperature (24–28°C) (Whiteside, 1975). If the disease occurs in the spring flushes, it may develop rapidly depending on weather conditions, and eventually, the fruit symptoms become more severe. The conidia formed in the lesions are disseminated by air mixed in raindrops and spread to distant places. Leaves are most susceptible just after emergence and are resistant to infection by the time they reach half expansion. Fruits are susceptible to infection for 6–8 weeks after petal fall (Timmer et al., 2000). However, if the environmental conditions are conducive, infections may occur even after 3–4 months of petal fall.

17.7.4 Pathogenicity and virulence

The citrus scab pathogen, *E. fawcettii*, can infect commercial species of citrus such as sweet orange (*C. sinensis*), lemon (*C. limon*), grapefruit (*C. paradisi*), many tangerine, Clementine and mandarin lines (*C. reticulata*), Satsuma mandarin (*C. unshiu*), and their hybrids, as well as species used as rootstocks such as sour orange (*C. aurantium*), rough lemon (*C. jambhiri*), and Cleopatra mandarin (*C. reshni*) (Hyun et al., 2009). The sweet orange scab pathogen, *E. australis*, is mainly distributed in South America and usually affects only sweet orange fruits. Scab diseases do not occur in the Mediterranean citrus-growing areas. It is also common to find *E. fawcettii* infecting ornamental citrus and rootstocks like calamondin (*C. madurensis*) and *P. trifoliata*. It is not common to find *E. fawcettii* in Persian limes, kumquats, pummelos, citrons or limes (Chung, 2011).

17.7.5 Disease management

Growing citrus cultivars resistant to scab diseases may be ideal, but there are some practical limitations. Therefore, for the control of scab diseases it is recommended to remove the affected plant tissues to reduce the pathogen inoculum (Timmer et al., 2004). Since new conidia are generated in the next growing season in lesions of twigs, leaves and remaining fruit infected in the previous season, it is necessary to remove these diseased tissues as much as possible at harvest and by pruning. Even when there is a small amount of diseased tissue left, the disease can develop quickly under favorable weather conditions. Thinning and pruning allow exposure to sunlight and enhance air movement, reducing the humidity in the orchard, and thereby minimizing disease development. Overhead irrigation should not be used on susceptible varieties for production of fresh fruit because it greatly increases the amount of infection.

When scab is severe and there is substantial inoculum carryover from the previous season, fungicides should be applied before the spring flush is one-fourth expanded. If the disease develops in the spring flush, fungicide sprays should be applied more than two times after petal fall until the fruit becomes resistant to infection. However, in the case of sweet orange scab, it is not necessary to control it in the spring flush. Strobilurin fungicides are of the most effective, reaching over 98% of disease control. Copper fungicides can be also used, but they are less effective. Benomyl, and sterol-biosynthesis-inhibiting fungicides were also reported effective for scab control, but their use is currently restricted in many countries. Fungicides with the same mode of action should not be used consecutively because resistant isolates can develop. When sprayed on fruits for scab control, fungicides can be chosen also to control black spot, melanose or other diseases simultaneously (Timmer et al., 2004).

17.8 Mal secco disease caused by *Plenodomus tracheiphilus*

17.8.1 Introduction

Mal secco is a vascular disease of lemon (*C. limon*) and other species of the genera *Citrus*, *Fortunella*, *Poncirus*, and *Severinia* and their hybrids, caused by *Plenodomus tracheiphilus* (= *Phoma tracheiphila*). The pathogen enters the tree through wounds in leaves, branches, and roots and colonizes the xylem vessels, causing wilt and leaf drop and ultimately dieback of twigs and branches, hence the Italian name "mal secco" (dry disease) (Cutuli et al., 1984; Perrotta and Graniti, 1988). Two other syndromes were described: "mal fulminante" (sudden death) and "mal nero" (black disease) due to root infections. "Mal secco" is most prevalent and severe on lemon, lime (*C. aurantifolia*), citron (*C. medica*), and bergamot (*Citrus* × *bergamia*). The disease was first described by Petri (1929), but symptoms were already observed in the Greek islands of Chios and Poros (Perrotta and Graniti, 1988). During the years its devastating effects have affected almost all the lemon producing countries of the Mediterranean Basin (EFSA, 2014a).

17.8.2 Disease symptoms

The typical symptoms consist of red discolored strands in the xylem of stems, leaf vein chlorosis, wilt, and leaf drop and ultimately dieback of twigs and branches (Fig. 17.8A). Symptom expression starts as slight discoloration of the primary and secondary veins, leaf and shoot chlorosis, wilting of leaves, leaf drop, and twig and branch dieback (Migheli et al., 2009). The petioles often remain for a period after leaves drop. Newly infected shoots, branches, and trunks show a salmon-pink or reddish-brown color, along with gum production, within the xylem vessels. On the withered twigs, immersed, flask-shaped, or globose pycnidia develop under the epidermis and emerge as black points within lead-gray or ash-gray areas. Frequently, a sprouting of shoots from the base of affected branches and rootstock suckers is observed. When infection starts at the base of a twig or branch the disease progresses more rapidly, whole shoots or branches wilt followed by leaf and fruit drop.

17.8.3 Infection process

Infection occurs by conidia that are produced in pycnidia on withered twigs or by phialoconidia produced by phialides formed on "free" hyphae grown on exposed wood surfaces, wounded plant tissues, and within the xylem elements (Migheli et al., 2009; Nigro et al., 2011). Conidia are usually dispersed by water-splash and wind-blown rain. Conidial germination may occur at temperatures between 5°C and 30°C with an optimum at 25°C. Temperatures above 30°C inhibit mycelial growth but do not kill the pathogen. Warm moist conditions are favorable to the infection and disease development. The pathogen enters susceptible host tissues (leaves, branches, twigs, and roots) by both conidia and mycelium through wounds/injuries. Penetration through stomata has never been demonstrated (Zucker and Catara, 1985). Once in the host, the fungus reaches the lumen of the xylem and then spreads systemically, mostly upward, by the conidia transported in the xylem sap.

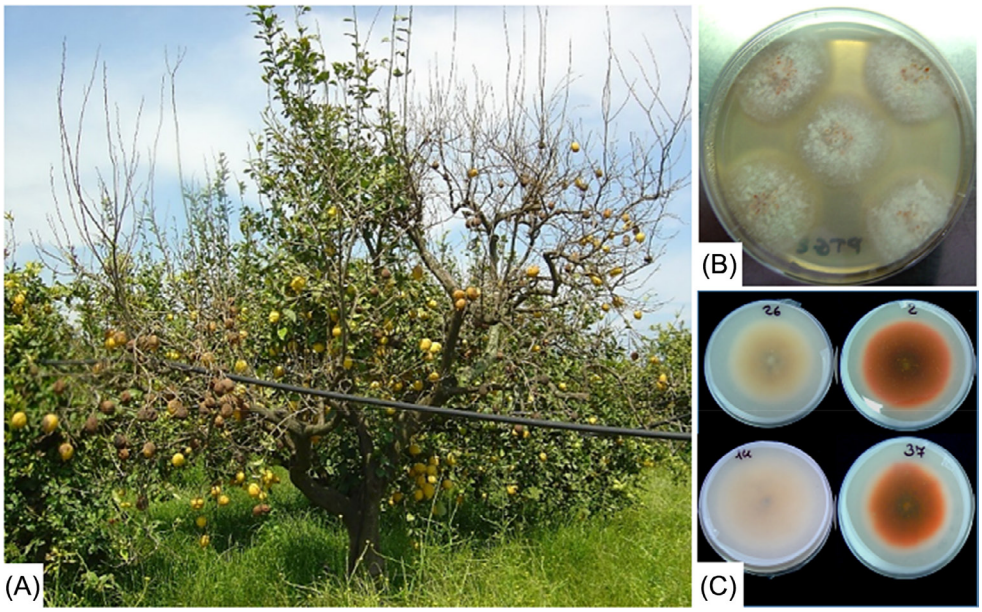


FIG. 17.8 Dieback of twigs and branches (A) caused by mal secco disease; (B) *Plenodomus tracheiphilus* colonies arising from infected wood; and (C) colonies with different pigmentation.

The infection period depends on local climatic and seasonal conditions. Any rainy period is conducive for infection provided the inoculum is present. The length of the incubation period varies depending on the climatic conditions, the age of the host and the form of the disease. In the case of "mal secco" it ranges between 2 and 7 months, whereas in "mal nero" it may last for several years. Near saturation humidity and temperatures between 20°C and 25°C are favorable for the production of phialides on hyphae present in wounds and leaf scars. *P. tracheiphilus* can survive as mycelium and conidia within pycnidia on infected leaves, twigs, and branches of susceptible citrus hosts. Infections occurring in lemon seeds do not have any epidemiological role. The survival potential of the pathogen in infected host plant debris lying on the soil varies between 30 days to 1 year depending on the soil type. The number of propagules in the soil decreases during the dry season (Russo et al., 2011).

17.8.4 Pathogenicity and virulence

Symptom distribution and severity are correlated with xylem colonization as in other tracheomycotic diseases (Migheli et al., 2009; Nigro et al., 2011). Conidia could be passively transported by the xylem sap flux reaching rapidly to twigs located far from the inoculation point. *P. tracheiphilus* is able to produce both hydrolytic enzymes and toxins. Toxin transport via-xylem leads to leaf chlorosis. The systemic invasion by the pathogen causes the impairment of water transport in the plant and consequent wilt associated with progressive clogging of the xylem by both gums and mycelium. Later, digestion of the walls of neighboring conduits leads to an easier and faster pathway for fungal diffusion. The lysis of pit membranes provides circumstantial evidence for the production of pectic enzymes by the fungus during the colonization process, which may act as virulence factors.

Culture filtrates of *P. tracheiphilus* induce wilting and chlorosis in lemon and nonhost plant cuttings such as tomato. Two nonselective phytotoxic glycoproteins of 93 and 60 kDa (called Pt60) (generally referred to as malseccin) have been extracted from culture filtrates of *P. tracheiphilus* (Migheli et al., 2009). Both toxins were able to reproduce symptoms of leaf vein chlorosis when injected into citrus leaves. Pt60 toxin has been isolated also from the leaves and the xylem of naturally infected lemon trees showing symptoms of the disease including leaf vein chlorosis, the typical salmon-orange wood discoloration, and leaf drop. One of the most recent studies pointed out the synchronous presence in the fungus of hydrolytic enzymes, toxic compounds, oxidative stress inducers, and membrane transporters. The contemporary differential ability to modulate the lipoperoxidative pathway in the host could play a key role in the interaction between *P. tracheiphilus* and its hosts.

Chromogenic and nonchromogenic variants of the fungus based on the production in the culture of red pigments were described; the nonpigmented isolates showed reduced virulence (Fig. 17.8B and C) (Migheli et al., 2009). ITS sequencing and most DNA fingerprinting techniques failed to show substantial genetic variability among isolates of the fungus, among them f-AFLP was the most discriminant (Nigro et al., 2011; Russo et al., 2011).

17.8.5 Disease management

P. tracheiphilus is of quarantine concern therefore strict regulation is adopted from pathogen-free countries (EPPO, 2019). As the introduction of *P. tracheiphilus* is a serious threat to lemon-growing areas, restrictions on the movement of citrus propagating material are mandatory. Therefore, the certification of plant materials is a critical step in disease control (EFSA, 2014b). Conventional and real-time PCR-based diagnostic methods are available based on SCARS or ITS primers (Nigro et al., 2011). The protocols are amenable for in planta detection as well as for fungus identification. The detection of the fungus in the soil was also achieved.

Where the disease is present, it is important (and part of mandatory protocols) to prune withered shoots, remove suckers, followed by burning of pruned branches to reduce inoculum (Perrotta and Graniti, 1988; Nigro et al., 2011). Pruning is performed in late spring and early summer when symptoms are more easily recognized. If the main trunk is not colonized by the fungus, removal of the diseased branches can limit spread and sometimes cure the tree of the disease. Fungicide applications are recommended in the nursery. In the orchards, applications of copper compounds or other authorized fungicides (e.g., mancozeb) are useful during the rainy season, after hailstorms, frost damage, and pruning. Many efforts have been made for the establishment of lemon cultivars that combine resistance with agronomic and qualitative characteristics. Currently, no citrus cultivars/clones or rootstocks resistant/tolerant to *P. tracheiphilus* infection that have competitive yields and satisfactory bioagronomic features are available for use on a large scale to control the disease in infested citrus-producing areas.

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