Chapter 12
Ceratocystis Wilt Pathogens: History and Biology—Highlighting *C. cacaofunesta*, the Causal Agent of Wilt Disease of Cacao

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Abstract *Ceratocystis* is a genus of ascomycete fungi that includes aggressive pathogens of economically important plants worldwide. This fungus is the causal agent of Ceratocystis wilt disease and canker disease, which often kills the plant causing major losses in agricultural production. In the last two decades, emerging diseases related to *Ceratocystis* infections have been greatly increased. Ceratocystis wilt of cacao is caused by *C. cacaofunesta,* one of the three well-established host-specific pathogens in the genus. Ceratocystis wilt of cacao has caused sporadic epidemics in the Americas, but its importance is often underestimated. Furthermore, the disease represents a serious threat to the world's cacao production due to the risk of pathogen spread. Silvicultural practices in cacao agroforests, the marketing of seeds, and cacao grafting in association with a minimal knowledge of the biology of the pathogen effectively contributed to this threat. This chapter explores the controversial taxonomic and evolutionary history of the genus *Ceratocystis* as well as the biology of *C. cacaofunesta*.

12.1 Introduction

Ceratocystis pathogens are major economic problem on a wide variety of more than 40 economically important agricultural crop plants worldwide, including *Theobroma cacao*. This genus of fungi is easily disseminated by human activities and shows increased rates of dissemination due to the ease with which infected plants

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can contaminate crop management systems. Like the other ascomycota, *Ceratocystis* is a group of confusing taxonomy due to its conserved morphology and the surprisingly broad host and geographic range. In the last few decades, the use of molecular tools has resolved many of the complexities of the *Ceratocystis* species, as well as the movement of these pathogens worldwide with the emergence of new host-specialized lineages. However, their taxonomic status still requires careful consideration. Species identification continues to be a challenge for the group and the development of new methods for this purpose is essential.

In the recent years, the recognition of its economic importance has resulted in intense scientific interest for the *Ceratocystis* group, which has led to an increase in publications concerning this genus and a high demand for new species descriptions. The association of Ceratocystis wilt disease with the ecological interaction of these fungi and bark beetles has also been the focus of many recent studies.

C. cacaofunesta is the causal agent of Ceratocystis wilt of cacao and is one of the few species of this genus with well-established species status and host specificity and representing a significant risk to cacao production. The risk is especially high in the Americas, where it was first reported followed by its rapid spread throughout the continent. In this chapter, we organized information concerning the biology and pathogenic features of all *Ceratocystis* stricto sensu species, but with a special focus on *C. cacaofunesta*.

12.2 Impact of Ceratocystis Wilt Disease on Agriculture

The genus *Ceratocystis sensu stricto* Ellis & Halst. includes many aggressive plant pathogens that cause wilt, canker stain diseases, and tissue rot on a wide variety of perennial and economically important agronomic crop plants worldwide (Kile 1993). Specially, *C. fimbriata* has had recent sporadic epidemics and is particularly important as the causal agent of coffee canker disease in *Coffea* plantations in Colombia and Venezuela (Pontis 1951). Coffee canker disease has become one of the most important threats to coffee production in Colombia, causing losses equivalent to US$174 per hectare and decreasing the coffee tree density from 1000 trees/ha to 950 trees/ha, on average (Castro-Caicedo 1998). Also in Colombia, Ceratocystis wilt disease in citrus has caused significant losses, estimated between 1 and 10 % of the total production (Borja et al. 1995). In Brazil, *C. fimbriata* isolates vary greatly in their aggressiveness to different hosts. However, they do not have a strong host specialization (Harrington et al. 2011). *C. fimbriata* causes lethal, wilt-type diseases or cankers in important plantation or orchard crops, such as *Gmelina arborea*, rubber tree (*Hevea brasiliensis*), inhame (*Colocasia esculenta*), and figs (*Ficus carica*) (Ferreira et al. 2010; Harrington et al. 2011). Brazilian isolates of *C. fimbriata* have caused important losses in *Mangifera* plantations (Rossetto and Ribeiro 1990; Ribeiro et al. 1995). However, in Brazil one of the major economic hosts of *C. fimbriata* is *Eucalyptus*. Ceratocystis wilt disease of eucalyptus was described for the first time in 1997 in Bahia causing mortality of 40 % in clonal

plantations (Ferreira et al. 1999; Ferreira 2000), and in a short period of time, the disease spread to other Brazilian States (Ferreira et al. 2006). Genetic analyses of isolates of *C. fimbriata* suggest that Brazilian genotypes have now been moved to other regions of South America, Africa, and Asia in infected eucalyptus cuttings (Roux et al. 2000; Barnes et al. 2003; Van Wyk et al. 2006b, 2012; Ferreira et al. 2010, 2011; Harrington et al. 2011).

Ceratocystis wilt diseases have also caused important economic losses in Europe and Asia. *C. platani* causes canker stain of plane trees (*Platanus orientalis*) and is a major threat to European plantations (Engelbrecht et al. 2004). It has killed hybrid plane trees in Italy since the 1930s (Engelbrecht et al. 2004) and *C. platani* has spread to France, Spain, Switzerland, and Greece (Panconesi 1999; Tsopelas and Angelopoulos 2004). Actions to remove the susceptible plane tree hybrids in Europe have been estimated at a cost of 200 million euros (RCE 2014). In Asia, Ceratocystis wilt disease has caused significant losses on *Punica* plantations in India (Somasekhara 1999) and *Ipomoea* production in China and Japan (Clark and Moyer 1988).

Oak Wilt disease, caused by *C. fagacearum*, has received much research attention because the epidemics originate in forest ecosystems in the United States (Juzwik et al. 2008). However, the latest taxonomic study of this group showed that this pathogen no longer belongs to the *Ceratocystis* strict genus (De Beer et al. 2014).

Ceratocystis wilt disease of *Theobroma cacao* was reported for the first time in Ecuador in 1918 (Rorer 1918). The lack of references about the disease suggests that it was confined to Ecuador until the 1950s, when reports arose of the disease in neighboring countries (Fig. 12.1). Also during the 1950s, Ecuador saw an increase in the disease particularly on the cacao trees type "criollo" with loses totaling 65,000 trees (Desrosiers 1957; Delgado and Suárez 2003). In Colombia, in 1956, Ceratocystis wilt of cacao affected more than 5000 trees (Arbelaez 1957). In the same year in Venezuela, the disease destroyed over 20 % of existing plants, estimated to be about 120,000 cacao trees (Malaguti 1956). In the late 1950s, Trinidad and Tobago reported the death of 2153 trees infected with the disease (Iton 1960).

Since the 1930s, Brazil has occupied a prominent position among the largest cacao producers in the world (Sauer 1993). Specifically, the state of Bahia was responsible for about 75 % of the national cacao production. In 1989, *Moniliophthora perniciosa*, the causal agent of witches' broom disease was introduced in Bahia drastically affecting the Brazilian role in the world cacao market (Pereira et al. 1989). Different strategies were followed in an attempt to control witches' broom disease. Breeding studies of cacao led to the identification of the Theobahia variety, which showed high productivity and resistance to witches' broom disease (Monteiro et al. 1995). Local producers replaced the cacao production clones with Theobahia grafts (Souza and Dias 2001). However, the hybrid showed a high susceptibility to Ceratocystis wilt of cacao (Silva and Luz 2000). In 1997, this disease was found for the first time in Bahia in seedlings propagated for grafting (Bezerra 1997). According to official data, no farms were lost due to Ceratocystis
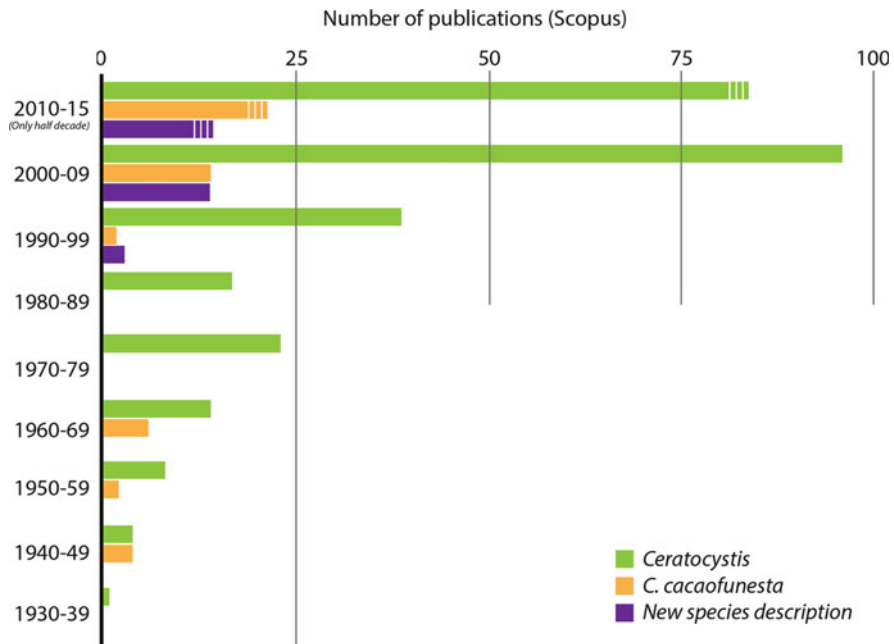
Number of publications (Scopus)

Fig. 12.1 Increase in *Ceratocystis* scientific publications. Data were obtained by searching "*Ceratocystis*" in the title, abstract, and keywords of the Scopus scientific bibliography databank. The searches were further filtered using "*cacaofunesta*" and "*sp. nov.*" It is noteworthy that the most recent data referenced in the last 5 years already comprised more than 80 % of the total number of publications from the previous decade

wilt of cacao, although the disease affected 24 % of the plantations in the region (Ram et al. 2004; Silva and Luz 2000). Figure 12.1 shows impact of Ceratocystis wilt of cacao on scientific research based on the number of publications and how the disease in Bahia changes the research focus after the disease was verified in the 1950s. Estimates suggest that millions of cacao trees have been killed due to Ceratocystis wilt of cacao although no quantifiable figures exist (Dand 2011).

Ceratocystis wilt of cacao is reemerging as an important disease because agricultural practices (like intensive pruning) facilitate the spread of the fungus into cuttings and seeds of cacao (Engelbrecht et al. 2007a). This is a serious threat to the producing countries, especially to African countries (Côte d'Ivoire and Ghana) which are the top cacao producers in the world (ICCO 2013). Recently, Mbenoun reported the isolation of two *Ceratocystis* spp. (*C. ethacetica* and *C. paradoxa*) in *Theobroma cacao* (Mbenoun 2014; Mbenoun et al. 2014a). Also, in Colombia, new *Ceratocystis* species were identified (*C. colombiana* and *C. papillata*) in coffee, cacao, and citrus trees (Van Wyk et al. 2010b).

The increase in the identification of new disease caused by *Ceratocystis* species found in cacao mirrors emerging *Ceratocystis* spp. diseases worldwide. Figure 12.1 shows that in the last 5 years there are already an approximately equal number of

references describing new species of *Ceratocystis* compared to all of the reported in the 2000s. In Brazil, cupuassu (*Theobroma grandiflora*) was recently described as a new host of *C. fimbriata* (Oliveira et al. 2013a). Pakistan, which is a major producer and exporter of mango (Al Adawi et al. 2013b), has reported large production losses of mango resulting from a Ceratocystis wilt disease caused by *C. manginecans* (Kazmi et al. 2005; Al Adawi et al. 2006). Ceratocystis wilt caused by *C. manginecans* has also devastated the mango industry in Oman (Al Adawi et al. 2006, 2013a; Van Wyk et al. 2007b). Recently, in Indonesia two new diseases of *Acacia mangium* were described and found to be associated with *C. manginecans* and *C. acaiivora* (Tarigan et al. 2011). Furthermore, in Australia, two new species of *Ceratocystis* were described on Eucalyptus (Nkuekam et al. 2012). Africa was the last continent where Ceratocystis wilt disease was officially described (Morris et al. 1993; Wingfield et al. 1996), however, the number of *Ceratocystis* pathogens identified in this region increased suddenly in the last year (Mbenoun 2014; Mbenoun et al. 2014a; Nkuekam et al. 2008, 2013; Heath et al. 2009; Van Wyk et al. 2012).

The data suggest that major new threat to agriculture lies in the emergence of new *Ceratocystis* species and adaptation to new hosts and new geographic regions. In 2003, Baker and coworkers described 31 host species that are infected by *Ceratocystis* species and today the number of hosts has increased to 42 (CABI 2015). This alarming situation has attracted the attention of the scientific community, resulting in intense research of Ceratocystis wilt diseases (Fig. 12.1).

12.3 Taxonomy

Taxonomic Status of the Genus Ceratocystis

The taxonomic history of the genus *Ceratocystis* is complex and has been subject of considerable study (De Beer et al. 2014; Harrington 2000; Wingfield et al. 1993). During the course of more than one century, it has been intertwined with several other genera like *Ophiostoma*, *Leptographium*, and *Raffaelea*; consequently the genus has been subjected to many name changes (Wingfield et al. 1993; De Beer et al. 2013).

Ceratocystis was originally described in 1890 by Halsted as the causal agent of black root rot of sweet potatoes (*Ipomoea batatas* Lam.). However, the complete mycological description of the pathogen was given by Halsted and Fairchild (1891). The specimen deposited by Halsted in 1891 was named *Ceratocystis fimbriata*, which is also the type species of this genus. This specimen was described as a parasitic fungus with a black perithecia and globose bases with long necks (sexual fruiting bodies) and sticky ascospore masses at their tip (Halsted 1890; Upadhyay 1981; De Beer et al. 2014). These morphological features are similar to features for species of the genus *Ophiostoma,* consequently *Ceratocystis* was once included in

this genus (De Hoog and Scheffer 1984). Like *Ceratocystis*, *Ophiostoma* also has an association with ambrosia beetle species and includes some plant pathogens like *O. ulmi* (Buisman), which causes an important vascular wilt disease of conifers (Harrington 2013; Brasier 1987). In the past, this similarity in morphology, ecology, and their association with insects for these two genera, resulted in confusion and they have constantly been treated collectively (De Beer et al. 2014).

A summary for the name change and synonym for species of *Ceratocystis* is provided here: The list illustrates the confusion and overlapping nomenclature for this genus.

Ceratocystis (ceratos=horn and cyst=pouch or sac)

Ceratocystis fimbriata, Ellis & Halst., In: Halsted, New Jersey Agric. Coll. Exp. Sta. Bull. 76:14. 1890.

=*Sphaeronaema fimbriatum* (Ellis & Halsted) Sacc, Syll Fung. 10:125. 1892.

=*Ceratostomella fimbriata* (Ellis & Halsted). Elliott, Phytopathology 13:56. 1923

=*Rostrella coffeae* Zimmermann. Meded. Lds. Pl. Tuin, Batavia. 37:32. 1900.

=*Ophiostoma fimbriatum* (Ellis & Halsted) Nannf, Svenska Skogsv Fören. Tidskr. 32:408. 1934.

=*Endoconidiophora fimbriata* (Ellis & Halsted) R.W. Davidson, J. Agric. Res. 50:800. 1935.

=*Ophiostoma variosporum.* Von Arx, Antonie van Leeuwenhoek. 1952.

=*Ceratocystis moniliformis f. coffea* (Zimm.) C. Moreau, Bull. Sc. Minist. France Outre-Mer 5:424. 1954.

Type Species: Ceratocystis fimbriata *(Ellis & Halsted)*

The close relationship between species of *Ceratocystis* and *Ophiostoma* has given rise to the general name "ophiostomatoid fungi," a collective reference including the later described genus *Ceratocystiopsis* (Hausner et al. 1993a; Spatafora and Blackwell 1994). *Ceratocystis sensu lato* has also been used collectively for all the ophiostomatoid fungi (Wingfield et al. 1993). These genera have been subjected to many taxonomic studies designed to demarcate the ophiostomatoid fungi (De Beer et al. 2013).

Different data including morphological, ecological, and phylogenetic analysis based on DNA sequencing have shown clear evidence that these three genera are distinct and phylogenetically unrelated (De Hoog and Scheffer 1984; Wingfield et al. 1993; De Beer et al. 2014). *Ophiostoma* and *Ceratocystis* were separated based on their different anamorph states (De Hoog and Scheffer 1984). The asexual states of *Ceratocystis* species were previously classified in the genus *Chalara* and more recently in *Thielaviopsis* (Paulin-Mahady et al. 2002). In contrast, *Ophiostoma* have anamorphs in *Leptographium* Lagerberg and Melin (Minter et al. 1983). Species of *Ceratocystis sensu stricto* can also be distinguished from other ophiostomatoid fungi by not having cellulose and rhamnose in their cell walls

(De Hoog and Scheffer 1984; Marín Montoya and Wingfield 2006) and being sensitive to the antibiotic cycloheximide (Harrington 1981). Furthermore, members of *Ceratocystis* do not have obligate associations with ambrosia beetles, in contrast to the more specialized ecological relationship between species of *Ophiostoma* and ambrosia beetles (Harrington 2013). Most species of *Ceratocystis* produce fruity volatile metabolites that attract a wide range of insects (Goheen and Hansen 1993; Harrington 2013). Several authors have proposed that the morphological similarity between Ophiostoma fungi and *Ceratocystis* has resulted from convergent evolution of these structures, in response to their similar niches and especially to the adaptation of insect dispersal (Spatafora and Blackwell 1994).

The first study in this group using molecular tools was published by Hausner et al. (1993b), presenting phylogenetic support for the separation of ophiostomatoid fungi using the large subunit ribosomal RNA gene. Afterward, studies based on analysis of partial sequences from the small subunit ribosomal (rDNA) and β-tubulin genes have revealed that not only do *Ceratocystis* and *Ophiostoma* represent two distinct genera, but they also fall into two separate orders (Spatafora and Blackwell 1994). *Ceratocystis* was accommodated into the order Microascales, family Ceratocystidaceae, while *Ophiostoma* and *Ceratocystiopsis* now resides in a separate order, the Ophiostomatales in the Sordariomycetidae subclass, which is phylogenetically distinct from *Ceratocystis* (Spatafora and Blackwell 1994; De Beer et al. 2014).

Complexes of Species Within Ceratocystis

Despite the effort that has been made to delimit the genus *Ceratocystis* from other *Ophiostoma* fungi, additional work is required to investigate and define the species within this genus. Much confusion still exists regarding the taxonomy of *Ceratocystis* species. Taxonomic studies have shown high genetic diversity in this group leading to propose the use of species complexes within the genus (Webster and Butler 1967a; Harrington et al. 1998; Wingfield et al. 1996; Witthuhn et al. 1998; Harrington 2000). Traditional biological species concepts are often inappropriate for fungi, especially in species complexes like this, with high rates of asexual reproduction and several morphologically similar species. Therefore, Harrington and Rizzo (1999) proposed a species concept for fungi based on diagnostic phenotypic characters within monophyletic lineages of organisms, as populations or lineages with unique phenotypic characters, such as morphology and host specialization.

Recent phylogenetic studies using rDNA sequence data (Wingfield et al. 1994, 1996; Witthuhn et al. 1998; Barnes et al. 2003), mating-type genes (Witthuhn et al. 2000; Harrington et al. 2002), host specialization, isozyme variation (Engelbrecht and Harrington 2005; Johnson et al. 2005), phylogenetic analysis of the rRNA ITS regions (highly variable internal transcribed spacers) (Van Wyk et al. 2007a, 2009, 2010a; Harrington et al. 2011), and microsatellite DNA regions

(Barnes et al. 2001) have reinforced the hypothesis that *Ceratocystis* species, which have been contemplated as a single species, actually represent complexes of cryptic species (Marín Montoya and Wingfield 2006). In 2000, Harrington analyzed rDNA-ITS sequences, partial sequence of MAT-2 mating-type genes, and interfertility tests to isolate groups of *Ceratocystis*. The results suggested that the genus *Ceratocystis* is formed by a complex of species mainly defined by geographical origin and host-associated lineages (Harrington 2000; Engelbrecht and Harrington 2005). *Ceratocystis* species were separated into a North American complex, Latin American complex, and an Asian complex. In the Latin America complex, two specific host species were clearly recognized, *C. cacaofunesta* (Engelbrecht and Harrington 2005) and *C. platani* (Engelbrecht and Harrington 2005), which appear to have adapted to infect *Theobroma cacao* and *Platanus occidentalis* (sycamore), respectively (Engelbrecht and Harrington 2005; Harrington et al. 2014). This hypothesis has been supported using microsatellite and minisatellite markers, which grouped the host-specialized isolates in separate clades in accordance with their respective host and origins (Barnes et al. 2003). It has become evident that some of these species, in fact, represent a complex of several host-specialized forms and cryptic taxa, which suggests that host specialization may be an important factor in recent speciation events within the genus (Harrington et al. 2014).

Afterward, Wingfield et al. (2013) delimited five different taxonomic groups into the genus *Ceratocystis* supported by phylogenetic and ecological analyses. These included species of the *C. fimbriata* complex, *C. coerulescens* complex (Munch) Bakshi, *C. moniliformis* complex (Hedcock) Moreu, as well as the *Thielaviopsis* and *Ambrosiella* complexes (Wingfield et al. 2013; De Beer et al. 2014). Each of these groups includes several morphologically cryptic species (Engelbrecht and Harrington 2005; Mbenoun et al. 2014b). For example, species in the *C. fimbriata* complex include important plant pathogens like *C. albifundus*, a virulent pathogen of *Acacia mearnsii* in Africa (Roux and Wingfield 2013); *C. cacaofunesta*, a pathogen of cacao in South America (Engelbrecht et al. 2007a); *C. platani*, an invasive pathogen of Platanus trees in Europe (Ocasio-Morales et al. 2007); and *C. manginecans* that has devastated mangos (*Mangifera indica*) in the Middle East (Van Wyk et al. 2007b; Tarigan et al. 2011).

The total number of species described in this genus is not clear due to its complicated taxonomic history with other fungi. However, in 2013, Seifert and coworkers listed 68 species in this complex (Mbenoun et al. 2014b; De Beer et al. 2014). In fact, during the last decade, there has been an increase in the number of species described for this genus (Van Wyk et al. 2007a).

The most recent work published by De Beer et al. (2014), using multiple gene regions (60S, LSU, MCM7) as well as morphological and ecological data for 79 species, residing in *Ceratocystis sensu lato* including the *C. fimbriata*, *C. coerulescens*, and *C. moniliformis* complexes, defined these species complexes into discrete genera. The new genera are **Ceratocystis** Ellis & Halst (previously *C. fimbriata* complex), **Chalaropsis** Peyronel (previously *Thielaviopsis* complex) **Endoconidiophora** Munch (previously *C. coerulescens* complex part 1), **Thielaviopsis** Went (previously *C. paradoxa* complex), and **Ambrosiella** Brader

ex Arx & Hennebert (previously Ambrosiella), with two taxonomic novelties **Davidsoniella gen. nov,** Z.W. de Beer, T.A. Duong, & M.J. Wingf, 2014 (previously *C. coerulescens* complex part 2) and **Huntiella gen. nov,** Z.W. de Beer, T.A. Duong, & M.J. Wingf, 2014 (previously *C. moniliformis*) (De Beer et al. 2014; Fourie et al. 2015). In this study all species in the *C. fimbriata* complex remained in the *Ceratocystis* genus because the genus is typified by the type species *C. fimbriata*. The genus currently includes 32 species (see Fig. 12.2) and many of them are important plant pathogens of angiosperm trees or root crops (Kile 1993; Engelbrecht and Harrington 2005; Van Wyk et al. 2013; Roux and Wingfield 2013; De Beer et al. 2014). Some previous *Ceratocystis* species continue in an uncertain taxonomic position as *C. adiposa*, *C. radicicola*, and important pathogens like *C. fagacearum* and *C. paradoxa*. Those species remained outside the three major phylogenetic groups defined by Wingfield et al. (2013). However, for now, these species still remain in the *Ceratocystis* genus until more data and additional species discoveries can define their position (Wingfield et al. 2013; De Beer et al. 2014).

As previously mentioned, significant progress has been made to define and delimit *Ceratocystis* at the genus and species level. Many studies and the development of novel taxonomic tools, especially the ones used to characterize DNA sequence variation, will be required to clarify the internal structure for the genus. In some cases, species boundaries for *Ceratocystis* are very clear, for example in the cases of the tree pathogens *C. platani*, *C. cacaofunesta*, and *C. albifundus* (Wingfield et al. 1996; Engelbrecht and Harrington 2005), while in others, the distinction at the species level is still being debated (Fourie et al. 2015; Harrington et al. 2014). It is important to employ new methodologies and studies that can help to delineate the taxonomy and systematic nature of this important genus. Future studies and new data on whole genome sequences for *Ceratocystis* species could clarify the taxonomy of this group. At this moment, the complete genome is available for only some species like *C. fimbriata*, *C. moniliformis*, and *C. manginecans* (Fourie et al. 2015).

Ceratocystis cacaofunesta *the Causal Agent of Ceratocystis Wilt of Cacao as a Newly Described Species*

Scientific name: *Ceratocystis cacaofunesta* Engelbrecht and Harrington 2005. Mycologia 97:64. 2005.

Synonyms:

=*Ceratocystis fimbriata* (Ellis & Halsted 1890)
=*Ceratostomella fimbriata* (Ellis & Halsted 1890)

Species	Host	Geographic range	Putative dispersor	References
C. acacivora	Acacia mangium Eucalyptus spp.	Indonesia China		Tarigan et. al. (2011) Chen et. al. (2013)
C. mangenicans	Mangifera spp. Acacia spp. Prosopis cineraria Dalbergia sissoo	Pakistan Oman Indonesia	Hypocryphalus mangiferae	van Wyk et. al. (2007a) Tarigan et. al. (2011) Al Adawi et. al. (2013a, b) van der Nest et. al. (2014)
C. curvata	Eucalyptus deglupta	Ecuador		van Wyk et. al. (2011)
C. mangicola	Mangifera indica	Brazil	Hypocryphalus mangiferae	van Wyk et. al. (2011)
C. mangivora	Mangifera indica	Brazil	Hypocryphalus mangiferae	van Wyk et. al. (2011)
C. polyconidia	Acacia meamsii	South Africa		Heath et. al. (2009)
C. eucalypticola	Eucalyptus spp.	South Africa		van Wyk et. al. (2012)
C. fimbriata	Ipomoea batatas Mangifera spp. Eucalyptus spp. Annona spp. Colocasia esculenta Punica granatum Eriobotrya japonica Hernania sp. Coffea arabica Xanthosoma sp. Gmelina sp. Cassava, Crotalaria, Passiflora edulis	Brazil Colombia Costa rica USA New Zeland Papua N Guinea Suriname China Japan	Hypocryphalus mangiferae	Halsted and Fairchild (1891) Baker et. al. (2003) Ploetz (2003) Silveira et. al. (2007) Yu et. al. (2011) Ferreira et. al. (2013) Firmino et. al. (2013) Wilken et. al. (2013) Li et. al. (2014) Zhang et. al. (2014) Harrington et. al. (2015)
C. fimbriatomima	Eucalyptus spp.	Venezuela		van Wyk et. al. (2009)
C. colombiana	Coffea arabica Citrus spp. Schizolobium parahybum	Colombia		Marin et. al. (2003) van Wyk et. al. (2010a)
C. platani	Platanus spp.	USA Greece Italy France	Platypus cylindrus	Engelbrecht and Harrington (2005) Souliotti et. al. (2015)
C. papillata	Coffea arabica Theobroma cacao Schizolobium parahybum Citrus x tangelo Citrus limon Annona muricata	Colombia		van Wyk et. al. (2010)
C. ecuadoriana	Eucalyptus deglupta	Ecuador		van Wyk et. al. (2011)
C. neglecta	Eucalyptus spp.	Colombia		Rodas et. al. (2008)
C. cacaofunesta	Theobroma cacao	Brazil Costa rica Colombia Ecuador Venezuela	Xyleborus spp.	Engelbrecht and Harrington (2005) Engelbrecht et. al. (2007b) Ambrosio et. al. (2013)
C. polychroma	Syzygium aromaticum	Indonesia	Hexamitodora semivelutina	van Wyk et. al. (2004)
C. ficicola	Ficus carica	Japan		Kajitani and Masuya (2011)
C. obpyriformis	Acacia meamsii	South Africa		Heath et. al. (2009)
C. pirilliformis	Eucalyptus spp.	Australia South Africa	Brachypeplus depressus	Barnes et. al. (2003) Roux et. al. (2004) Nkuekam et. al. (2009) Nkuekam et. al. (2013)
C. tsitsikammensis	Rapanea melanophloeos	South Africa		Nkuekam et. al. (2012)
C. zambeziensis	Combretum imberbe Acacia nigrescens Schotia brachypetala	South Africa		Mbenoun et. al. (2014b)
C. diversiconidia	Terminalia ivorensis	Ecuador		van Wyk et. al. (2011)
C. albifundus	Acacia meamsii Acacia exuvialis Protea cynaroides	South Africa Uganda Kenya	Carpophilus spp.	Heath et. al. (2009) van der Nest et. al. (2014)
C. variospora	Quercus prinus	USA		Johnson et. al. (2015)
C. caryae	Carya cordiformis	USA		Johnson et. al. (2015)
C. smalleyi	Carya spp.	USA		Johnson et. al. (2015)

Fig. 12.2 Host species, geographic range, putative insect dispersers for species maintained in *Ceratocystis* genus after systematic revision of De Beer et al. (2014). The phylogenetic hypothesis shown on the *left* is composed of branches with strong statistical support (*dark red*) obtained by Fourie et al. (2015) and branches with medium support (*light red*) obtained by De Beer et al. (2014)

Common names: Ceratocystis wilt, cacao canker, Ceratostomella disease, *Xyleborus–Ceratostomella* complex, cacao wilt, Ceratocystis canker, mort subite (French), mal del machete (Spanish), necrosis del cacao, la llaga macana

del tronco del cacao, murcha de Ceratocystis em cacaueiro, mal do facão (Portuguese),

Taxonomic position

Kingdom: Fungi; Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Ceratocystidaceae; Genus: *Ceratocystis*

The causal agent of Ceratocystis wilt of cacao, *Ceratocystis cacaofunesta,* was described in 2005 by Engelbrecht and Harrington as a new species within the Latin American clade of the *C. fimbriata* species complex. They differentiated *C. cacaofunesta* from other strains of the *C. fimbriata* complex by its specificity to infect *Theobroma cacao*, as well as phylogenetic data (ITS-rDNA sequences), intersterility (sexual incompatibility) tests, and some morphological differences like the rare production of doliform (barrel-shaped) conidia and minor differences in size of perithecial bases and ascospores (Engelbrecht and Harrington 2005; Engelbrecht et al. 2007b). Engelbrecht and Harrington employed a species concept defined by Harrington and Rizzo in 1999 (see complexes of species within *Ceratocystis above*). In previous studies, Baker and coworkers (2003) showed host specialization in a "pull" of lineages in reciprocal inoculation experiments on cacao (*Theobroma cacao*), sweet potato (*Ipomoea batatas*), and sycamore (*Platanus* spp.) which form three distinct ITS genotypes (Baker et al. 2003). In this work, besides *C. cacaofunesta,* the other identified species within the *C. fimbriata* species complex was *C. platani* (Harrington et al. 2014). Subsequent studies with microsatellite alleles (Steimel et al. 2004) and mating studies confirm the species boundaries between *C. fimbriata*, *C. platani*, and *C. cacaofunesta* (Engelbrecht and Harrington 2005; Ferreira et al. 2010).

C. cacaofunesta is a specialized pathogen on a single host, causing Ceratocystis wilt of cacao (Engelbrecht and Harrington 2005; Engelbrecht et al. 2007a, b; Ploetz 2007; Harrington et al. 2011; Ambrosio et al. 2013). The pathogen may be indigenous to the Americas, because this disease has been reported only in Central and South America (Harrington 2000; Baker et al. 2003; Engelbrecht and Harrington 2005; Engelbrecht et al. 2007a; Ferreira et al. 2010), where it is commonly referred as "Mal del machete" from the association of infections with contaminated machete wounds. The first report of this disease occurred in Ecuador in 1918, and initially the causal agent of this disease was identified as *C. fimbriata* (Rorer 1918). At that time, it was considered an unimportant disease because the variety of cacao prevalent in Ecuador was resistant to Ceratocystis wilt (Wood and Lass 2001). Then in 1957, Desrosiers (1957) reported a more virulent form of the fungus at the Pichilinque Research Station in Ecuador on the Criollo cacao varieties, which was less severe on Trinitario and Forastero cacao cultivars (Wood and Lass 2001). Thenceforth this disease has been reported in many countries of South America; Colombia, Brazil, Guyana, Peru, Trinidad and Tobago, and Venezuela; in Central America; Costa Rica, Guatemala, and México and in the Caribbean island of Haiti; where it has affected a significant number of cacao trees (Arbelaez 1957; Thorold 1975; Pontis 1951; Soria-Vasco and Salazar 1965; Engelbrecht et al. 2007a). Actually, this is one of the most important "emerging" diseases of cacao in Central and South America (Engelbrecht et al. 2007a; Ploetz 2007).

12.4   Life History

Morphology

The *Ceratocystis fimbriata sensu stricto* complex includes several morphologically cryptic species (Baker et al. 2003; Harrington 2000) and means that morphological variations in the genus are subtle, almost indistinguishable while its members are unable to interbreed (Engelbrecht and Harrington 2005). The main morphological differences between species lie in some aspects associated with reproductive structures (Engelbrecht and Harrington 2005). Although *C. fimbriata* is the type species of the complex, in this section we will describe the morphology of *Ceratocystis cacaofunesta,* the pathogen of *Theobroma cacao*.

Ceratocystis cacaofunesta (= *Ceratocystis* spp.) forms a filamentous tallus. The vegetative mycelium consists of septate, branched, and smooth-walled hyphae (Engelbrecht et al. 2004). In culture, initially the mycelium is hyaline and then it gradually becomes darker (Baker et al. 2003; Engelbrecht and Harrington 2005). This is because of the accumulation of melanin pigment in hyphal walls (Harris and Taber 1973). The fungus exhibits the three reproductive modes described in filamentous ascomycetes: vegetative propagation and asexual and sexual reproduction (Mbenoun 2014). The following paragraph describes the morphology of the reproductive structures of *C. cacaofunesta*.

Asexual spores (conidia) are produced during the anamorphic state of the fungus by conidiophores through ring wall building (Minter et al. 1983) (Fig. 12.3g). Those endoconidiophores develop from branches of the vegetative hyphae and arising scattered or in clusters, up to 40–295 mm long, including the basal cells and 2.0–8.0 mm wide at the base. They are 0–12 septate with phialide at the top (Engelbrecht and Harrington 2005). The most common phialide is lageniform, hyaline to light brown with 12–85 mm long, 2.0–9.0 mm wide in the middle and 2.0–6.5 mm wide at the tip (Engelbrecht and Harrington 2005). Also, wide-mouthed phialides with tones ranging from hyaline to light brown can be observed, albeit rarely, borne near to the base of perithecia. *C. cacaofunesta* mainly produces two types of conidia: (1) cylindrical endoconidia which are the most abundant spore forms produced by the fungus and (2) aleurioconidia which are produced by specialized conidiophores. Endoconidia are unicellular, hyaline to light brown, smooth, mainly cylindrical with truncated ends, straight, and biguttulate. Their dimensions range from 2.5 to 5.0 mm (Engelbrecht and Harrington 2005). Endoconidia are exuded through a cylindrical collarette on the phialide borne in chains of variable lengths; aleurioconidia are oval, thick walled and pigmented in olive-brown tones to allow for long-term survival. A third type of spores, the doliform conidia, could be produced by *C. cacaofunesta* but is rarely produced (Engelbrecht and Harrington 2005). Doliform conidia are produced from wide-mouthed phialides, often remaining in chains, and hyaline to light brown (Engelbrecht and Harrington 2005). Asexual and vegetative reproductions give rise to progeny that are genotypically identical to their parents.
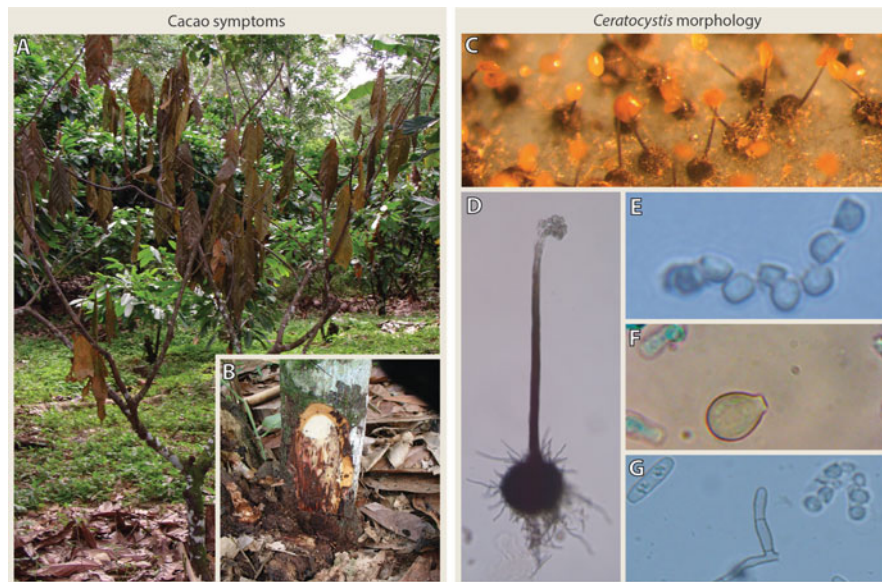
Fig. 12.3 Symptomatology of Ceratocystis wilt of cacao and morphological features of the causal agent *C. cacaofunesta*. (**a**) A dying cacao tree showing the typical symptoms: wilt and necrotic leaves, particularly in this disease the wilted leaves remain attached to the branches for several weeks after the tree dies. (**b**) Vertical cross section of cacao tree infected with *C. cacaofunesta* showing the dying regions of parenchyma in dark shadows. (**c**) Ascomata of *C. cacaofunesta* on Malt-Agar. Dark-brown perithecia with a globose base immersed in the substrate and orange sticky ascospore masses emerging from their necks. (**d**) Perithecium with masses of ascospores emerging from the ostiolar hyphae. (**e**) Hat-shaped hyaline ascospores. (**f**) Brown and globose aleurioconidia. (**g**) Mycelium and cylindrical endoconia of *C. cacaofunesta*

The teleomorphic state (sexual) of *C. cacaofunesta* is observed after 48 h of growth in cultured media with the beginning of the perithecium's rostrum formation (Santos et al. 2013). Sexual spores (ascospores) are produced in large numbers within asci (meiosporangia) which are organized inside the perithecium (ascocarp) (Fig. 12.3e). Ascospores are hyaline, single-celled, and are characteristically hat-shaped (Engelbrecht and Harrington 2005) (Fig. 12.3f). Perithecia can be produced superficially or embedded in the substrate. The perithecium is globose (95–305 μm wide and 100–275 μm tall) and dark brown to black. It has a long (310–1010 μm), straight and at the base (12–25 μm wide at the tip) neck which also is dark brown to black (Engelbrecht and Harrington 2005). The ostiolar hypha is divergent, light brown to hyaline, nonseptate, smooth-walled and ranging 30–125 mm long with an ostiole at the end. Each ascus consists of groups of eight haploid spores. When the ascospores are fully mature, the asci walls disintegrate and the spores are enveloped in a gelatinous mass (Engelbrecht and Harrington 2005; Chong 1961). Within the perithecium hydrostatic pressure is generated pulling the mucilaginous matrix up the perithecium neck and out of the ostiole.

Spores accumulate in a white to yellowish droplet at the tip of the perithecial neck (Hunt 1956; Upadhyay 1981) (Fig. 12.3d). It has been proposed that this is an adaptation to insect dispersal because this allows the spores to adhere to the insect exoskeletons (Malloch and Blackwell 1993). The ascospores typically germinate within 20–25 h on water agar with one or several germ tubes emerging from the top of the spore, thus establishing haploid mycelia (Santos et al. 2013).

Life Cycle

The life cycle of *C. cacaofunesta* will be explored here as a representation of all *Ceratocystis* species (Fig. 12.4). This is because the overall cycle is common throughout the genus, only varying in some minor aspects of the interaction with different plant hosts.
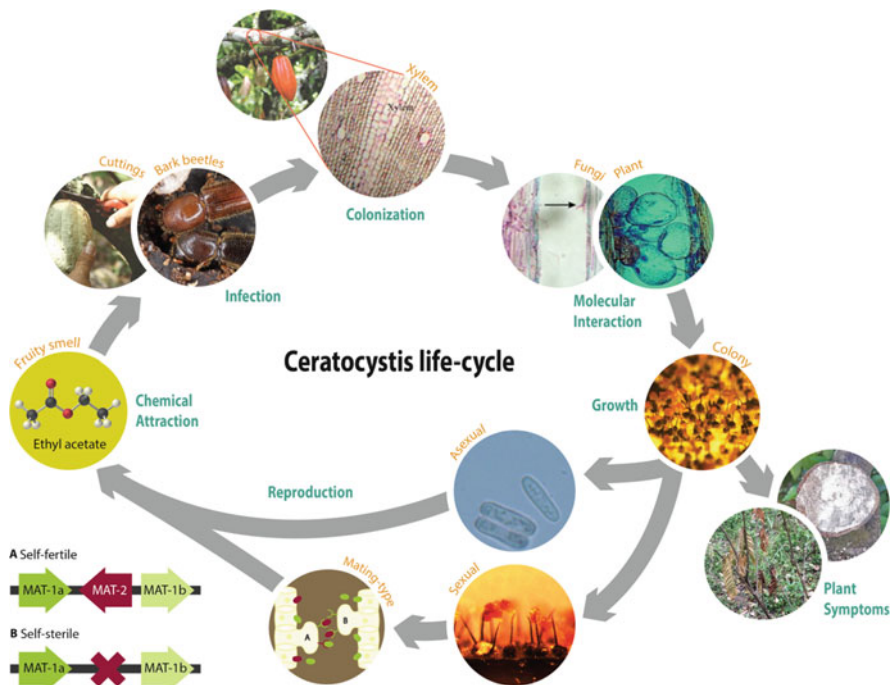

Fig. 12.4 *Ceratocystis* life cycle represented by *C. cacaofunesta* cycle on cacao hosts. Each stage is discussed in detail in the text

Infection

Since *Ceratocystis* species are primarily xylem pathogens, wounds to the xylem are the main infection pathway for most species of the genus (Harrington 2000). However, *Ceratocystis* also causes root infections via soil-borne aleuroconidia through root anastomoses between neighboring trees (Rossetto and Ribeiro 1990; Harrington 2009). This infection strategy has been well described for isolates of *C. fimbriata* infecting mango, eucalyptus, sweet potato and for isolates of *C. platani* (Engelbrecht and Harrington 2005; CABI 2015; Harrington 2000; Johnson et al. 2005).

C. cacaofunesta enters cacao tissue passively through wounds on the stem, caused by either insects (natural causes), often bark beetles (Goitía and Rosales 2001; Mazón et al. 2013), or infected cutting tools (Malaguti 1956; Saunders 1965; Santos et al. 2013). The fungus infects the xylem parenchyma cells reaching the xylem vessels in the stem (CABI 2015). *C. cacaofunesta* moves through the host into the secondary xylem (Malaguti 1952). In cacao, roots of infected trees by *C. cacaofunesta* often show necrosis suggesting that it is also a soil-borne pathogen (Engelbrecht et al. 2004, 2007a).

Recently, Araujo et al. (2014) showed that isolates of *C. fimbriata* obtained in Brazil are able to colonize all stem tissues of mango. Firmino et al. (2013) showed for the first time that *C. fimbriata* can infect fruit of *Passiflora edulis* (Firmino et al. 2013). The fact that *Ceratocystis* pathogens are able to infect other plant tissues, beyond the xylem vessels, has led to suggest that *Ceratocystis* are non-true vascular pathogens (Viégas 1960; Araujo et al. 2014).

Germination and Colonization

Since *C. cacaofunesta* (like all *Ceratocystis* species) is delivered directly to the targeted host tissue, it does not form plant penetration structures like appressoria or haustoria. Once inside the plant tissue, chlamydospores (aleurioconidia) germinate, probably triggered by exudates of the host plant (Ioannou et al. 1977). Spores of true vascular wilt pathogens, like *Verticillium* and *Fusarium*, germinate and grow well in in vitro xylem fluids (Kessler 1966). Amino acids and sugars contained in the xylem have been associated to wilt disease resistance (Booth 1969; Singh et al. 1971). However, studies of the nutritional conditions for the *Ceratocystis* fungi in the xylem are missing. In the natural host, fungal colonization is restricted to a small area around the wound site (Johnson et al. 2005). On susceptible host, *Ceratocystis* fungi colonize extensively the pith parenchyma in a radial direction in the stem tissues of plants (Clérivet et al. 2000).

Histopathological studies of *C. cacaofunesta*—*T. cacao* in resistant and susceptible seedlings showed that fungal spores grow well in both genotypes and produce perithecia (Santos et al. 2013). However, different patterns of fungal colonization

were observed. Tissues from the resistant genotype showed minimal fungal colonization, high amounts of non-germinated conidia and few perithecia without masses of spores, whereas, the susceptible genotype was highly colonized. In addition, in the susceptible (CCN 51) clone, the fungus produced numerous perithecia, releasing ascospores at 4 h after inoculation (Santos et al. 2013). The authors observed atypical smaller conidia protruding from the pits during the early stages of colonization and suggested that this is the reason for the rapid distribution of the fungi (Santos et al. 2013).

Fungi–Plant Interactions

A common mechanism of tree's resistance to pathogens is based on the ability to restrict them to a few cells (Duchesne et al. 1992). Host resistance in response to wilt pathogens infection includes preformed and induced mechanisms (Duchesne et al. 1992). The host-induced reaction includes the production of gel, gum, and tyloses which operates as occluding structures (Duchesne et al. 1992). These structures serve to limit the colonization of the vascular tissue by the pathogen (Duchesne et al. 1992). Ouellette and Rioux (1992) showed that this response is effective in blocking the advance of the pathogen if it is produced in advance of fungal ingress or during the colonization process. On the other hand, the accumulation of these compounds in xylem vessels leads to the obstruction of water and nutrient transport (Duchesne et al. 1992). Consequently, the branches wilt and die, compromising the whole tree (Clérivet et al. 2000). In addition, host responses include the deposition of phenolic compounds in the parenchyma cells adjacent to the xylem vessels in an attempt to impede the xylem vessel colonization by the wilt pathogens (Shi et al. 1992; Hall et al. 2011).

The cacao responses, both resistant and susceptible, to *C. cacaofunesta* infection included (1) discoloration of primary walls of infected xylem vessels and the surrounding parenchyma cells; (2) mobilization of polyphenolic in parenchyma cells; (3) translocation and accumulation of starch in the xylem; and (4) production of gums and tyloses. The main difference between genotypes was the intensities of those responses being more pronounced in the susceptible varieties (Santos et al. 2013).

C. cacaofunesta is a necrotrophic fungus. Necrotrophs are species that extract nutrients from dead cells killed prior to or during colonization (Stone 2001). Typically, diverse phytotoxic compounds are deployed to induce cell necrosis and cause leakage of nutrients (Dickman and de Figueiredo 2013). Phytotoxins have been implicated in a number of diseases caused by *Ceratocystis* spp. Ceratoplatanin (CP) is the phytotoxin best characterized in *Ceratocystis*. Ceratoplatanin occurs in filamentous fungi performing different functions like allergen in humans, elicitor of plant defenses, and inducer plant resistant (Pan and Cole 1995; Hall et al. 1999; Rementeria et al. 2005; Seidl et al. 2006; Zaparoli et al. 2009; de O Barsottini et al. 2013; Baccelli et al. 2014). The first member of

this family functionally described was a protein of *Ceratocystis platani* (Pazzagli et al. 1999). CP was found in both the fungal culture supernatants and the cell wall of the fungus. The proposed role for CP located in the cell wall is, similar to hydrophobins, to allow the attachment of hyphae to hydrophobic surfaces during the formation of aerial structures (Pazzagli et al. 1999). However, the monomers act as a toxin or elicitor inducing necrosis in tobacco cells (Pazzagli et al. 1999, 2001, 2004; Boddi et al. 2004; Scala et al. 2004). Certainly, other phytotoxic compounds may be produced by the fungus and an analysis of fungal genome sequences could elucidate this point. Wilt symptoms in cacao infected by *C. cacaofunesta* could be due to toxins in combination with vessel occlusion (Santos et al. 2013).

Plant Symptoms

Ceratocystis pathogens cause diverse diseases such as root and tuber rot, canker stain diseases, and vascular wilt (Kile 1993; CABI 2015). Infected trees may die over a period of months or years (Juzwik et al. 2011). Within *Ceratocystis*, there are many different genetic strains that vary in their aggressiveness and resistance host (Harrington et al. 2011). Thus, a plant can be infected with a mild strain and exhibits moderate symptoms or a plant can be infected with a severe strain and be killed suddenly. Either way, often these diseases are fatal for the plant. Other typical symptoms of cacao wilt disease is the black- brown discoloration of the vascular tissue, this symptom is particularly visible after the plant has shown advanced stages of wilt (Santos et al. 2013). The internal infected woody tissue shows a brown red or purplish color decreasing in intensity toward the healthy tissue (Iton and Conway 1961; Wood and Lass 2001).

The classical symptoms of the Ceratocystis wilt of cacao are leaf chlorosis and wilt within 2–4 weeks after the infection (Fig. 12.3c). Leaves remain adhered to the plant for weeks even after the death of the plant (Silva et al. 2004; Delgado and Suárez 2003; Chong 1961). Internally, there occurs vascular browning due the trapping of the conidia at the parenchyma cells surrounding the xylem vessels (Santos et al. 2013) (Fig. 12.3a, b). Host defense responses are the key to generating a containment barrier against the pathogen, as mentioned above. If the fungus is trapped at this time, the pathogen may be contained. It suggests that the restricted vessel occlusion could be a mechanism to contain the pathogen's spread in resistant genotypes (Talboys 1972). However, if conidia are released into xylem sap and disseminated, then a large number of vessels can be colonized and blocked concomitantly leading to the inability of the plant to transport the water resulting in wilt (Talboys 1972). In addition, phenolic compound deposition and necrosis of the vascular parenchyma cells contribute to wilt symptoms (Santos et al. 2013).

Santos et al. (2013) described the kinetics of the Ceratocystis wilt of cacao seedlings. Resistant and susceptible plants at 6 months of age were inoculated with a *C. cacaofunesta* spore suspension. Macroscopic symptoms of leaf chlorosis and

wilt were evident in the susceptible seedlings 7 days after inoculation. After 15 days, most of these plants were completely wilted (Santos et al. 2013).

Reproduction

Vegetative propagation occurs when an unspecialized mycelia section is detached from the main body of the thallus and forms an independent colony or mycelia mass. Asexual reproduction originates from numerous mitospores (conidia) which are sections of modified hyphae.

An early key step in sexual reproduction is mate type recognition (Ni et al. 2011). Following mating, cells undergo cell-cell fusion resulting in a dikaryotic state that prepares the cells for nuclear fusion and meiosis (Ni et al. 2011). Fungi have evolved systems to detect mating partners via mating type-specific peptide pheromones and receptors (Ni et al. 2011). The mating in ascomycetes is determined by a bipolar system consisting of two idiomorphics forms (MAT-1 and Mat-2) of the single locus (MAT-1). In heterothallic fungi, haploid hypha can't undergo sexual reproduction until it meets another compatible partner. The compatibility consists of the interaction of hyphae with different but complementary alleles at the mating locus. However, isolates of homothallic fungi are able to complete their sexual cycle independently, through self-fertile interactions with a single individual capable of sexual reproduction (Ni et al. 2011).

C. cacaofunesta, as other species, belonging to *Ceratocystis sensu stricto*, has a bipolar mating-type system (Harrington and McNew 1997). However, most of those fungi are homothallic by a unidirectional mating-type switching mechanism (Harrington and McNew 1997; Witthuhn et al. 2000). This switching of mating type occurs only in Mat-2 strains and it is irreversible. Harrington and McNew (1997) suggested that MAT-2 isolates may delete the MAT-2 gene and express MAT-1, being self-sterile. Therefore, selfings give rise to a mixture of both self-fertile (MAT-2) and self-sterile (MAT-1) offspring. Recently, Wilken et al. (2014) through genome analysis showed that the MAT-1 locus of *C. fimbriata* comprises three genes (MAT-1-1, MAT1-2, and MAT2-1) with their respective domains similar to other ascomycota MAT-genes, including the presence of introns in conserved positions. However, the MAT locus of *C. fimbriata* exhibits an atypical organization with gene rearrangements in the position of individual genes. Also, self-fertile isolates become self-sterile through the complete deletion of the entire MAT2 gene (3581 bp) and its flanking regions (1911 bp) from the MAT locus (Wilken et al. 2014).

MAT-1 and MAT-2 progenies of *Ceratocystis* species segregate in variable ratios ranging from 1:1 to 9:1 during a selfing event (Webster and Butler 1967a). However, several studies have reported that MAT-1 strains are less abundant that MAT-2 isolates in nature and grow slower (Wilken et al. 2014; Harrington and McNew 1997; Olson and Martin 1949). Harrington and McNew (1997) suggested that the decreased fitness of self-sterile offspring is a pleiotropic character

associated with the loss of MAT-2. Since MAT-2 encodes a transcription factor, its deletion could affect the expression of genes under its control. Another explanation is the occurrence of chromosome rearrangements (Harrington and McNew 1997). Therefore, the most common reproduction in *Ceratocystis* is selfing (MAT-2), limiting the genetic diversity of the group (Harrington et al. 1998). Far from being a disadvantage, this feature could facilitate rapid speciation by interruption of the gene flow. Thus, introduced populations could persist in an essentially clonal manner (Engelbrecht et al. 2007b). In this regard, the self-fertile to self-sterile switch could be a mechanism of the fungus to avoid the accumulation of deleterious mutations linked to selfing and clonal reproduction throughout obligate outcrossing (Gioti et al. 2012).

Production of Volatiles from Species of Ceratocystis

Many species of the genus *Ceratocystis* have demonstrated the ability to produce a wide variety of fruity aromas. These compounds are important in the life cycle of these fungi, because it attracts the insect vectors (Iton 1966; Engelbrecht et al. 2004). In his classification of the genus *Ceratocystis*, Hunt (1956) mentions different types of aromas produced by different species suggesting that volatile metabolic products might be used as taxonomic markers. In 1983, Sprecher and Hanssen identified several acyclic monoterpene alcohols, aldehydes, and acetates as metabolic products from cultures of *C. fimbriata*. Since then, many other monoterpenes and sesquiterpenes compounds have been reported in other *Ceratocystis* species (Lanza and Palmer 1977; Sprecher and Hanssen 1983). Furthermore, from these volatiles, various others terpenes with fruity or floral aromas have been identified and are being used for large-scale production of bioflavors (Krings and Berger 1998; Vandamme and Soetaert 2002). Various analytical techniques have been applied to the identification of these volatile compounds, including gas chromatographic (GC) and gas chromatography-mass spectrometry (GC-MSD) (Christen et al. 1997; Sanchez et al. 2002). Volatile compounds produced by species of *Ceratocystis* are thought to be important in the attraction of insects, but some of these compounds are phytotoxic and may have a role in host–pathogen interactions (Tabachnik and DeVay 1980; Engelbrecht et al. 2004).

12.5 Evolutionary Biology

Ceratocystis spp. includes fungi that have primarily been shaped by the evolutionary forces resulting from their interaction with the plant hosts and with insect dispersers. Additionally, human activities may have complicated attempts to understand the pathosystem evolution by increasing their rates of evolution, creating new niches by introducing the pathogens to new areas and new hosts, and exerting

additional selection pressures (Ennos and McConnell 1995). In the next sections we will review the current knowledge of the ecology and evolution of *Ceratocystis* at the species and population levels of biological organization.

Genetic Diversity

C. fimbriata (Halsted and Fairchild 1891), the first described *Ceratocystis* species, was considered to have a broad geographic host range until the first proposition that the species actually consists of a complex of species (Webster and Butler 1967a, b). Only more recently, DNA-based techniques have made it possible to easily distinguish independently evolving lineages that might otherwise have been assigned to *C. fimbriata*. In the last year, many other fungal species initially described as members of the *Ceratocystis* genus were proposed to actually be separated into six different genera of the same family (De Beer et al. 2014). As a result, much of the diversity initially attributed to one species, *C. fimbriata,* is actually structured among several species.

Among the Brazilian group of *Ceratocystis fimbriata*, two host specific lineages were described as different species based on molecular phylogenies and intersterility tests: *C. cacaofunesta* and *C. platani* (Engelbrecht and Harrington 2005). Another four lineages from *Gmelina*, inhame, fig, and *Eucalyptus* were separated along host lines by analysis of microsatellite markers (Ferreira et al. 2010) and in the phylogenetic analysis of MAT1-2 gene loci but they are interfertile (Harrington et al. 2011). Mango isolates of *C. fimbriata* showed a wider molecular variation and did not group as a clade in the phylogeny of Brazilian lineages, having a lineage grouping with different lineages from other hosts and being interfertile with them all (Harrington et al. 2011).

Within *Ceratocystis* species, gene diversity is relatively low, around 0.2 for Nei's diversity for clone-corrected populations, but consistent for putative native populations of the fungus such as *C. albifundus* in South Africa (Roux et al. 2001; Barnes et al. 2005), *C. platani* and *C. cacaofunesta* in South America (Engelbrecht et al. 2004, 2007b), *C. fimbriata* populations on mango in Brazil (São Paulo and Rio de Janeiro) and on Eucalyptus planted in naturally infested soil, also in Brazil (Minas Gerais and Bahia) (Ferreira et al. 2010). Lower levels of genetic diversity and even clonal structures of *Ceratocystis* groups characterize populations that have undergone recent bottlenecks. These bottlenecks occur mainly in the form of founder effects of recently colonized areas/hosts resulting from human activity with one or a few pathogen variants. This was the case for *C. pirilliformis* and *C. eucalyptcola* (= *C. fimbriata*) from *Eucalyptus* in South Africa (Nkuekam et al. 2009; Van Wyk et al. 2012), for *C. fimbriata* from mango in the Brazilian state of Mato Grosso do Sul, for *Eucalyptus* in the Brazilian state of Bahia (Ferreira et al. 2010), for pomegranate wilt caused *by Ceratocystis fimbriata*, taro and lowquat in China (Huang et al. 2003), and for *C. mangenicans* from mango in Oman and Pakistan (Al Adawi et al. 2013b).

Among *C. cacaofunesta* populations, the gene diversity values for the Rondonian (Brazil) and Ecuadorian populations of *C. cacaofunesta* are similar to those of native populations of other homothallic *Ceratocystis* species. Populations from Costa Rica, Colombia, and Bahia (Brazil) showed lower gene diversity in nuclear and mitochondrial markers suggesting that these three populations on cacao are the result of introductions (Engelbrecht et al. 2007b). The Rondonian population is within the Upper Amazon center of diversity for *T. cacao* (Cheesman 1944; Bastos and Evans 1978; N'goran et al. 2000) and is possibly the source of the introduced populations in Bahia (Brazil), Costa Rica, and Colombia (Engelbrecht et al. 2007b). The Ecuadorian population may be native on other species of *Theobroma* and shows a higher genetic divergence from other populations not only based on molecular markers but also in the difficulty in obtaining hybrid progeny from strain pairings (Baker et al. 2003). Actually *C. cacaofunesta* have a restricted distribution, and is present only in the central and South America regions. However, potentially this pathogen could be moved to others regions with the major f risk being in areas like Africa and Asia, where the majority of cacao is produced.

The relatively low levels of overall gene diversity in *Ceratocystis* populations may be related to three important characteristics leading to limited outcrossing (Yu et al. 2011; Harrington et al. 2015). First, members of the *C. fimbriata* complex are homothallic due to unidirectional mating-type switching (Witthuhn et al. 2000). Second, their natural insect dispersers have a limited flight range (Harrington 2013). Third, the long-term dispersal is usually associated with human transport of infected plant tissues usually resulting in the introduction of only one or a few genetic variants of the pathogen (Ferreira et al. 2011, 2013).

Geographic Range and Dispersal

Nowadays, the *Ceratocystis* genus has a worldwide distribution (Fig. 12.5). There is a higher density of species and occurrences in the South American tropics suggest that this region is the ancestral range of *Ceratocystis* species. The diversity patterns described from genetic markers such as DNA fingerprints and microsatellite markers, mitochondrial sequences, and RFLP haplotypes can help to distinguish indigenous populations from introduced ones. There is strong evidence that members of *Ceratocystis* Latin American clade colonized other hosts and continents in vegetative cuttings taken from asymptomatic hosts (Engelbrecht et al. 2007b; Ferreira et al. 2010; Harrington et al. 2015), especially Eucalyptus cuttings from Brazil that were introduced into China as early as 1984 (Chen et al. 2013; Harrington et al. 2015). Among clones of Eucalyptus spp. that were widely planted in South China in the 1990s, were *E. urophylla* × *E. grandis* hybrids from Brazil and *E. urophylla* clones from Indonesia, where the ITS5 haplotype of *C. fimbriata* was identified in Eucalyptus and *A. mangium* (Harrington et al. 2014).

Ceratocystis-infected Eucalyptus was also introduced into South Africa probably from a Latin American lineage of *C. fimbriata* (Van Wyk et al. 2006b) where it
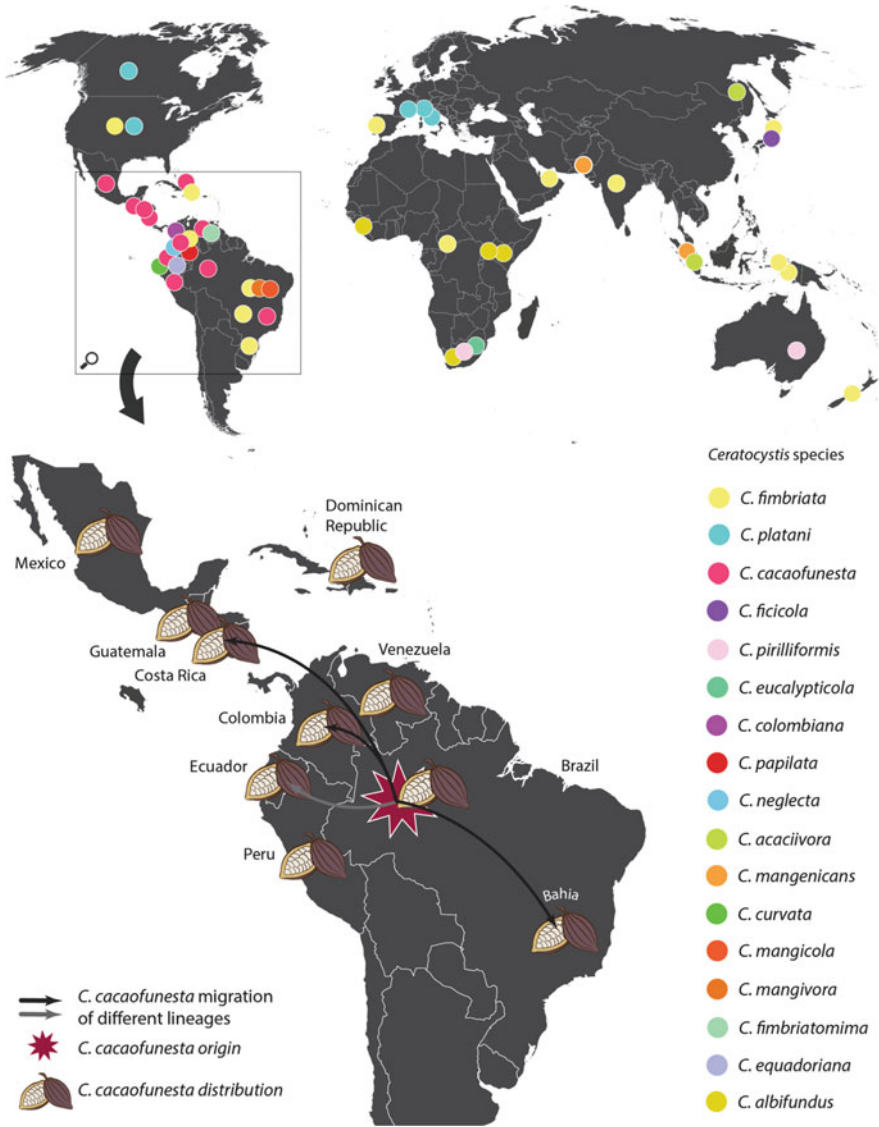
Fig. 12.5 Map of geographic distribution for *Ceratocystis*. Migration of *C. cacaofunesta* in Central and South America

diverged for reduced outcrossing and low diversity resulting from a founder effect and was recently proposed to be a new species, *C. eucalyptcolla* (Van Wyk et al. 2012). Another African lineage of *Ceratocystis* isolated in natural savanna trees, described as *C. thulamelensis*, is more closely related to South America lineages of *C. fimbriata* than to other South African lineages suggesting it was also introduced (Mbenoun et al. 2014b).

Ceratocystis platani is probably native to the southwest region of North America, but has spread throughout the Italian peninsula and too many areas of France (Ferrari and Pichenot 1976), Switzerland, and Spain (Ruperez and Muñoz 1980; Matasci and Gessler 1997). In 2003, the disease was first observed in plantings of *Platanus orientalis* in Greece, causing widespread mortality (Tsopelas and Angelopoulos 2004). Since then, *C. platani* has spread into the native range of *P. orientalis* in southern and north-western Greece causing massive epidemics and extensive ecosystem degradation in natural stands of *P. orientalis* (Ocasio-Morales et al. 2007; Tsopelas et al. 2011).

Insect–Pathogen Interactions

One of the reasons *Ceratocystis* was first considered an Ophiostomatoid fungus is the presence of previously described adaptations to insect dispersal, including the long-necked perithecium with passive discharge of adhesive ascospores from evanescent asci. However, those adaptations are considered a product of convergent evolution, i.e., evolving independently (Malloch and Blackwell 1993). Among *Ceratocystis s. l.* the ecological interactions with insects are mainly opportunistic and highly variable (Harrington 2009). Species initially attributed to the *C. fimbriata* complex are now known to have close associations, mainly with casual insects (Juzwik et al. 1998) mediated by the production of fruity aromas. These aromas are attractants for fungal-feeding insects (Lin and Phelan 1992; Kile 1993). Fungal-feeding insects such as drosophilid flies (Diptera) and nitidulid and scotilid beetles (Coleoptera) have been frequently associated with mycelial mats of *Ceratocystis,* but *Ceratocystis sensu stricto* usually do not have specific insect vectors (Kile 1993).

Nitidulid beetle transmission of *C. fimbriata* has been demonstrated with the *Populus* (Hinds 1972), *Platanus* (Crone 1963) and *Prunus* (Moller and DeVay 1968) isolates of the fungus. *Ceratocystis fagacearum*, the causal agent of Oak Wilt disease in North America has a known ecological relationship with nitidulid beetles. Many species of nitidulids inhabit *C. fagacearum* mats, but *Carpophilus sayi* and *Colopterus truncatus* appear to be particularly important vectors in the Upper Midwest and suggests that *C. fagacearum* may have coevolved with these vectors (Juzwik et al. 2004; Ambourn et al. 2005).

Some *Ceratocystis sensu lato* fungi, currently placed in the related genus *Ambrosiella* (De Beer et al. 2014), show an obligate relationship with Ambrosia beetles (Coleoptera: Scotilinae) (Engelbrecht and Harrington 2005). Ambrosia beetles cultivate ambrosiella fungi for feeding benefiting the fungi with a specialized transport to new host plants. Many of the ambrosia beetles have special spore-carrying sacs, called mycangia, and the fungal symbionts are transported in these sacs (Beaver 1989). Although ambrosia beetles are not common vectors of *Ceratocystis sensu stricto*, there are two descriptions suggesting that ambrosia beetles can vector *Ceratocystis*. The beetle *Hypocryphalus mangiferae,* largely associated with

infected mango trees in Pakistan and Oman (Masood et al. 2008), showed evidence that the beetle contributes to the transport *C. mangenicans* and xylem infect and spread (Masood et al. 2010). *Platypus cylindrus,* a previously described ambrosian beetle (Baker 1963), is developing a secondary mutualistic relationship with *C. platani* that infects *Platanus* spp. trees and was introduced in Europe (Soulioti et al. 2015).

Ambrosia beetle frass is also an important form of dispersal for the Latin American *C. platani* and *C. cacaofunesta,* which may be soil borne (Harrington 2009). Ambrosia beetles attack trees previously colonized by the pathogens (Ocasio-Morales et al. 2007) and the sawdust and fungal propagules expelled from the trees as the adult beetles clean their tunnels may be dispersed by wind or rain splash for relatively short distances (Iton and Conway 1961). On cacao, *Xyleborus* spp. (*Scolytidae*) selectively attack trees infected with *C. fimbriata* (former *C. cacaofunesta*), especially preferring trees with deteriorated bark (Saunders 1965). The adult female beetles bore into the tree perpendicular to the bark, usually at the base of the trunk (Iton and Conway 1961). Branching tunnels in which eggs are laid form horizontal planar galleries. *Ceratocystis* and other fungi may live within these galleries (Iton and Conway 1961).

Speciation Patterns

While lineages of *C. fimbriata* were deeply studied and host-specific lineages were described as new species, the hypothesis of host specialization in *Ceratocystis* has gained strength (Baker et al. 2003; Engelbrecht and Harrington 2005; Van Wyk et al. 2007a; Harrington 2009). Harrington (2000) used rDNA-ITS and MAT-2 mating-type gene sequences in a phylogenetic analysis that revealed three geographic clades within *C. fimbriata*, centered in Asia, North America, and Latin America. Within each geographic clade were several host-associated lineages that were considered possible cryptic species. Baker et al. (2003) identified two strongly supported lineages delineated by the ITS phylogenetic analysis that contained only isolates from cacao (*Theobroma cacao*) and sycamore (*Platanus* spp.). These two monophyletic lineages also showed intersterility, although not total, with each other and all Latin American lineages from different hosts. These lineages were later described as the species *C. cacaofunesta* and *C. platani*, with the aid of slightly divergent morphological characteristics (Engelbrecht and Harrington 2005).

In Indonesia, *Ceratocystis* spp. were first noted when *C. fimbriata* was reported in 1900 on *Coffea arabica* L. on the island of Java (Zimmerman 1900). Subsequently, various species of *Ceratocystis* have been reported from other hosts on many Indonesian islands. Examples include *C. fimbriata* from *Hevea brasiliensis* Müll.Arg in Sumatra, Kalimantan and Java (Leefmans 1934; Tayler and Stephens 1929; Wright 1925); *C. polychroma* M. Van Wyk, M.J. Wingf., and E.C.Y. Liew from *Syzygium aromaticum* (L.) Merrill and Perry in Sulawesi (Van Wyk et al. 2004) and *C. tribiliformis* M. Van Wyk and M.J. Wingf. from *Pinus merkusii*

Jungh. and de Vriese in Sumatra (Van Wyk et al. 2006a). In Japan, *C. ficicola* is phylogenetically more related to *Ceratocystis* species infecting Eucalyptus than to other lineages of *C. fimbriata* infecting fig hosts (Kajitani and Masuya 2011). The same pattern appears in *C. fimbriata* isolates in Colombia that are phylogenetically more related to *C. fimbriata* infecting other hosts in different countries (Marin et al. 2003). All of this evidence strongly suggests that host specialization may be an important force driving speciation in this genus.

Ecological speciation from host specializations appears to be a major route for the emergence of fungal plant diseases and some life-history traits of fungal plant pathogens can facilitate rapid ecological divergence (Giraud et al. 2010). *Ceratocystis* fits all the traits (Giraud et al. 2010). First, the strong disruptive selection imposed by different hosts requires rapid adaptation pathways. Although *Ceratocystis*, as many other fungal pathogens, shows a relatively low genetic diversity, it overcomes this limitation by the strategy of producing a large number of spores, increasing both the possibility of survival on a new host and increasing levels of adaptive variation created by mutation. Second, a high frequency of mating within a host occurs in *Ceratocystis* as a result of selfing and low dispersal distances by insects (Harrington 2009), creating pleiotropy between host adaptation and assortative mating. Third, the high frequency of asexual reproduction with rare events of sexual recombination (Witthuhn et al. 2000) prevents gene-flow among divergent lineages. Fourth, a low number of genes underlying the specificity of host–pathogen interactions are primarily evidenced by the comparison of *C. cacaofunesta* and *C. fimbriata* genomes that show a similarity of 98 % in gene composition (unpublished data).

Although many *Ceratocystis* species are concordant with the hypothesis of host specialization, worldwide migrations often induced by human activities opened the possibility of many allopatric speciations not necessarily associated with host-shifts. For example, *C. fimbriata* rapidly diversified in Indonesia with and without host-shifts. Among those diversifications, species of Eucalyptus infecting *Ceratocystis* in Indonesia have a single common ancestor with other geographic areas (Van Wyk et al. 2012) but diverged and have been described as different species worldwide: *C. atrox* (Van Wyk et al. 2007b) and *C. corymbiicola* (Nkuekam et al. 2012) from Australia, *C. pirilliformis* (Barnes et al. 2003) from Australia and South Africa, *C. neglecta* (Rodas et al. 2008) from Colombia, *C. fimbriatomima* (Van Wyk et al. 2008) from Venezuela, *C. zombamontana* (Heath et al. 2009) from Malawi, and *C. eucalypticola* from South Africa (Van Wyk et al. 2012). All of these species from Eucalyptus can be distinguished from each other based on phylogenetic inference and they have some morphological features that can be used to recognize them.

Harrington et al. (2011) also showed evidence that refutes the idea of host specialization for Latin American lineages of *C. fimbriata*. The Brazilian isolates appeared previously to be closely related to each other and to *C. fimbriata sensu stricto* based on the phylogenetic analysis of MAT1-2 sequences, which separated *Gmelina* isolates, inhame isolates, fig isolates, and Eucalyptus isolates from Bahia into groups (Engelbrecht et al. 2007b). If this genetic structure was being produced

by host specialization, one could expect isolates of one host to be particularly aggressive on cultivated hosts from the same family or genus of their native hosts. Harrington et al. (2011) studied the aggressiveness of isolates to other native and cultivated hosts and found that the variation in aggressiveness among isolates from a single host is as large as among hosts group of species. The explanation proposed is that the pathogen is not specialized to any particular host. Instead, an individual isolate has the potential for a wide range of aggressiveness to various hosts due to numerous quantitative genetic traits (Pariaud et al. 2009; Harrington et al. 2011).

12.6 Diagnosis and Control Strategies

Detection and Identification

In cacao and generally others *Ceratocystis* host trees like *Mangifera*, *Acacia* spp., *Prunus*, etc.; the principal sign of this disease is a wound caused by contaminated pruning tools and insect attacks. Another visible sign is that the leaves on a plant suddenly wilt, and the wilted leaves remain attached to the branches for several weeks after the tree dies (Engelbrecht et al. 2007a; Harrington 2000; Santos et al. 2013).

In plant material like stems and trunks, reproductive structures of the pathogen *C. cacaofunesta* are only visible at a very late stage of the infection process on mature trees. Santos and coworkers (2013) noted the absence of hyphae and conidia in inoculated susceptible and resistant cacao seedlings prior to wilting (Santos et al. 2013). In other species of *Ceratocystis* these structures can be detected by direct observation (naked eye) and usually recognized by their distinctive perithecia. Reproductive structures are difficult to observe in infected cacao trees. However, it is easy to obtain them, from parts of infected plants in a humid environment at temperature around 25 °C. The procedure usually results in ascomata production in a few days. In cases where there are no perithecia productions, pure culture of the fungus is required for reliable identification (Engelbrecht and Harrington 2005). Species of *Ceratocystis* can be isolated on potato dextrose agar, carrot agar, or malt extract agar (Harrington 2000). A carrot assay is a selective medium and has been used successfully for isolating *Ceratocystis*. The assay consists of placing a small piece of infected plant material between two fresh carrot disks that have been sterilized and incubate the plates at 25–27 °C in high humidity during 4–10 days. In this period, the production of fungal perithecia is initiated (Moller and DeVay 1968; Engelbrecht and Harrington 2005). Then, the ascospore masses from the perithecia are formed on the carrot disks are transferred to malt extract agar media for purification and subsequently storage in sterile water at room temperature (Castellani 1939) or in a cool chamber. It has been reported that the fungus can also be isolated from the body of ambrosia beetles using the

carrot assay (Goitía and Rosales 2001). *C. cacaofunesta* grows rapidly in malt extract agar. After approximately 24 h, a hyaline mycelium appears, and overtime the pathogen produces characteristic dark-brown perithecia (see above: morphology).

   C. cacaofunesta has been recently differentiated from other strains of the *C. fimbriata*. Unfortunately, we don't have yet a diagnostic protocol for its detection and identification as well as for its differentiation from other related fungal species. However, it is recommended to use the identification of the pathogen based on its culture and morphological characteristics reported by Engelbrecht and Harrington (2005). Molecular or serological diagnostic techniques have not been available, but there are microsatellite markers developed by Steimel et al. (2004) which had been used in population studies (Engelbrecht et al. 2007b) with intersterile populations and show unique microsatellite markers (Engelbrecht et al. 2007b). *C. cacaofunesta* can be confused with other species of *Ceratocystis*, principally with *Ceratocystis moniliformis,* but can be differentiated because the latter species is only weakly pathogenic (CABI 2001). *C. moniliformis* grows much more quickly on agar medium in laboratory conditions. In addition, its morphological characters are different: the perithecial bases of *C. moniliformis* have spine-like ornamentations and fungus fails to produce aleurioconidia (Hunt 1956; Harrington 2013).

Control Strategies

There is a wide variety of circumstances that hinder the implementation of controls throughout the multiple host ranges of *Ceratocystis* spp. However, most of the controls are expensive and may be harmful to the environment (Juzwik et al. 2011). Thus, prevention is a recommended approach for cacao farmers. Nevertheless, if the plantation is already infected or there is an imminent risk of infection it's recommended to implement mitigation measures (exclusion and eradication). Control measures like chemical and biological control and host resistant genotypes all target and interrupt the principal stages of the infection cycle of the fungus (Juzwik et al. 2011).

Avoidance Wound avoidance is the key to management diseases caused by *Ceratocystis* spp., including Ceratocystis wilt of cacao. Since it is a vascular pathogen, the recommended preventive measures are as follows: (1) Ensuring the health of new planting or grafting material since they can carry the pathogen from the nursery; (2) avoid causing wounds on trees; (3) prevent field personnel from climbing the trees during harvest or pruning during collection; (4) disinfect tools with any of the following products [sodium hypochlorite 5 %; formalin; benomyl (Benlate), thiabendazole (Mertect), and carbendazim (Bavistin and/or Derosal)], in doses of 4 cc or 1 ml/l of water; (5) pruning should be done preferably in the dry season and protecting wounds immediately after cutting with potassium permanganate 1 % or one of the above products; (6) a healing paste (pitch) can be applied to

large wounds and should be monitored periodically to ensure proper healing; and (7) periodical monitoring of the trees should be conducted, in order to detect early infections, so that subsequent sampling and treatment can be conducted.

Exclusion Nowadays, chemical treatments are used in packing materials to help control the spread of wood-boring insects. Thus, it could prevent the spread of *Ceratocystis* in insect droppings before reaching the final destination (FAO 2009). After pruning, when the wood is still moist is when the formation of mycelium begins beneath the bark (Gibbs and French 1980). Apparently, those conditions favor the fungal growth but it does not survive for long periods in the stem wood (Merek and Fergus 1954). A good practice recommendation is to clean the trunk with water and 8-hydroxyquinoline sulfate solution. Take care with the cutting implements and climbing ropes when they are used to avoid mechanical transmission. Finally, it is important to mention that some of the diseases caused by *Ceratocystis* spp. are categorized as an A1 quarantine pest by principal phytosanitary protection organizations around the world: the European and Mediterranean Plant Protection Organization (EPPO), Inter-African Phytosanitary Council (IAPSC), and the North American Plant Protection Organization (NAPPO).

Eradication The eradication of Ceratocystis wilt of cacao during local epidemics has been possible by following good sanitation practices (Harrington 2013). However, when an exotic disease epidemic arrives, it can be a difficult task to complete the eradication even in a limited area. This is because the high prevalence of the pathogen on non-host plants. Nevertheless, when the pathogen is quickly recognized, Ceratocystis wilt of cacao can be easily eliminated. All symptomatic trees, recently killed tree as well as neighboring trees must be removed in order to reduce disease prevalence and prevent the spread (Harrington 2013). After removal of all diseased trees, it is recommended to treat the diseased stumps with ammate, aresenite, or 2,4,5-trichloroethane-rophenoxyacetic acid (2,4,5-T). This treatment reduces the survival time of *Ceratocystis* (Merek and Fergus 1954). Additionally, farmers should take into account that by cutting diseased trees, there is a risk that the conidia will move to adjacent trees through root grafts. Therefore, root grafts between trees must be severed before felling the diseased trees.

The wood of the diseased trees can be utilized by the wood industry, burned, or buried, as long as the bark is removed and the wood is dry (French and Juzwik 1999). The tools and equipment that will be used to process the diseased trees must be disinfected with alcohol, sodium hypochlorite, or other chemicals. Treatments to the infected area should include the spraying of glyphosate or other herbicides to kill the root systems of infected plants and to further prevent the spread by root grafts (Harrington 2013).

Diseased trees should not be cut on windy days and their movement in and out of the affected area should be limited to minimize or prevent the spread of fungal infected particles such as sawdust. Additionally, the contaminated soil must be removed and disinfected. Physical and chemical methods are recommended, such as water vapor or by using products like metam sodium, dazomet, and

dicloropropen (Yuce et al. 2011). McCracken and Burkhardt (1979) reported that felling and removing from the area of diseased trees was not needed because *Ceratocystis* spp. had a short survival period. However, more recent studies have shown that *Ceratocystis* fungi can survive for long periods as aleurioconidia in sapwood. Therefore, the authors suggest removing the debris before the insects enter the timber and spread contaminated frass (Harrington 2013).

Protection Tree protection against *Ceratocystis* spp. involves fungicide injections, wound dressings, and trenching or other barriers. However, fungicide treatment can be problematic because vascular infected tissue is hard to be reached. In addition, the treatment may not be efficient when providing a single sustained dose. The high cost of the treatment and the tree injury caused during the injection process must be balanced with the likelihood that the treatment will reduce the risk of infection. Moreover, preventive treatments may only delay the onset of the disease. Fungicide treatment is not useful when the plant is already infected, as it does not stop the advance of the pathogen (Blaedow et al. 2010). Another disadvantage of triazols is the limited movement of the compound into the root system and short retention in the tree.

Walter et al. (1952) recognized that wound dressings commonly had sawdust with viable *C. platani* inoculum, which could survive for months. Fungicidal wound dressings have been recommended for the protection of pruning or other wounds from infection by *C. variospora* (DeVay et al. 1968) and other *Ceratocystis* spp. Latex paint is the recommended treatment because of its effectiveness and its lack of toxicity to the tree (French and Juzwik 1999; Camilli et al. 2007). Wound dressings also can inhibit the growth of *Ceratocystis* (Davis and Peterson 1973).

Reductions in root graft transmission by the use of root-free zones have been practiced for many years and can be highly effective in reducing losses caused by *Ceratocystis* (Bretz 1951; Gehring 1995; Cummings-Carlson et al. 2010; Juzwik et al. 2011). Generally, a trench is made to delimit the infected trees from healthy trees. The placement of the trench can be calculated based on the probability of disease spread (Bruhn et al. 1991). Generally, this protective practice involves two barriers, primary barrier using at least one ring of healthy trees beyond the symptomatic trees and a secondary barrier placed inside the first one that helps to protect the ring of healthy trees.

Host–Plant Resistance Plant resistant to *Ceratocystis* pathogens has been used successfully in *Mangifera* (Ribeiro et al. 1995; Rossetto et al. 1997), *Ipomoea* (Martin 1954), and *Crotalaria* (Ribeiro et al. 1995). In *Coffea,* three genotypes (MEG 639-601; MEG 639-617, and MEG 639-704) were selected from breeding programs (Castillo 1982; Castro-Caicedo and Cortina-Guerrero 2009; Castro-Caicedo et al. 2013). *Citrus* species have been shown to vary in their susceptibility to Colombian strains of *C. fimbriata* (Paez and Castano 2001). In contrast to other *Ceratocystis*, soil-borne populations of *C. fimbriata,* in Brazil this fungus can be quite diverse, and individual isolates vary greatly in aggressiveness to Eucalyptus and other hosts (Harrington et al. 2011). Four highly resistant clones of Eucalyptus

(Clones C15, C16, C17, and C18) are available to manage this disease (Zauza et al. 2004). Subsequently, Rosado et al. (2010) studied Ceratocystis wilt resistance by stem inoculations of *E. grandis* and *E. urophylla* genotypes and estimated the heritability and gains of selection in families derived from controlled interspecific crosses. As a result, a high degree of genetic control and low allelic dominance of the trait were found and five QTLs for Ceratocystis wilt resistance on Eucalyptus were mapped using microsatellite genetic maps for the interspecific, full-sibling family DGxUGL [(*E. grandis x E. dunnii* Maiden) x (*E. urophylla x E. globulus*)]. However, in both *E. grandis* and *E. urophylla*, there are clones that range from highly resistant to highly susceptible and the crosses of these two species show a high degree of heritability of resistance (Rosado et al. 2010). In cacao, most authors agree with the use of resistant genotypes as the more efficient strategy against *C. cacaofunesta* (Table 12.1). This is because the results are more stable and beneficial to the environment (Silva et al. 2004; Delgado 2003; Engelbrecht and Harrington 2005; Alarcon 1994; Guerrero 1975; Chong 1961). Desrosiers (1956) began finding differentiation between cacao varieties based on their susceptibility to infection with *Ceratostomella fimbriata*. During 1980s, international breeding programs for resistance to *Ceratocystis* were developed (Gardella et al. 1982; Ocampo et al. 1982) and resistant genotypes were identified under local conditions (Mata 1991; Delgado 2003). A classical method, developed by Delgado and Echandi (1965), has been employed to assess resistant genotypes. Basically, propagule suspensions are applied to branch sections to evaluate perithecial development. Ceratocystis wilt of cacao has gained great importance because much of the germplasm that has been selected for that is resistant to witches' broom and has proven to be susceptible to *Ceratocystis* (Engelbrecht et al. 2007a). Silva et al. (2004) reported the susceptibility of the clone IMC-67 to a *C. cacaofunesta*

Table 12.1 Different responses of cacao clones to *C. cacaofunesta* infection

Host response	Clones	References
Resistant	CEPEC 2002 (VB 1151), TSH 1188, PS 13:19 and CEPEC 2007 (VB 681)	CEPLAC collection; Oliveira et al. (2009)
Intermediate resistant	TSAN 792, PH 15, CP 44, CP 46, and ICS 1	Oliveira et al. (2009)
Susceptible	CCN 10, CA 1.4, SJ 02, CCN 51, and PH 16	Oliveira et al. (2009)
Resistant to both colonization and reproduction of the fungus	AMA-10, EBC-122, EBC-138, EBC-142, EBC-148, and LCT-EEN 73	Delgado (2003)
Resistant for avoid the pathogen reproduction	AMA-4, AMA-14, AMA-9, CUR-3, LCT-EEN 57, and LCT-EEN 382	Delgado (2003)
Resistant	POUND-12, EET-399 and EET-400	Delgado and Suárez (2003)
Resistant to Ecuadorian isolate, susceptible to Brazilian strain	IMC-47 and IMC-67	Delgado and Suárez (2003)

Brazilian isolated, which is concerning since this is one of the primary resistant clones used for rootstock to combat this disease.

Chemical Control Fungicides were first used with some success to treat tapping panels of *Hevea* (Chee 1970), in *Ipomoea* fields (Martin 1971) and in postharvest dips of *Ipomoea* roots (Daines 1971; Yang et al. 2000). Also, in *Ficus*, fungicides were used to control Ceratocystis wilt disease (Hirota et al. 1984). Stems of *Platanus* species have been injected with fungicides resulting in protection of the plant against *C. fimbriata* (Causin et al. 1995; Minervini et al. 2001). In cacao, because Ceratocystis wilt disease kills the plant, fungicides have the potential to prevent crop losses, but this is normally not economically feasible (Ferreira et al. 2013). Thus, sanitation is more effective for control of this disease. For example, disinfecting machetes and pruning tools between plants may help control the disease in *Platanus* (Walter et al. 1952 *Prunus* (Teviotdale and Harper 1991) and cacao and a 10 % solution of formalin is recommended to disinfect tools (FEDECACAO 2007). Moreover, all cacao cuttings from regions with Ceratocystis wilt disease should be grafted and grown under strict quarantine in a greenhouse before field release to ensure that they are free from *C. cacaofunesta* (Engelbrecht et al. 2007a).

Biological Control Several isolates of the genus *Trichoderma* have been tested in in vitro assays to evaluate the antagonistic potential to cacao pathogens such as *Moniliophthora perniciosa*, *Phytophthora palmivora*, and *Ceratocystis cacaofunesta*. Only *T. harzianum* and *T. pseudokoningii* have shown potential biocontrol in vivo suggesting that they could be used as control agents (Oliveira et al. 2013b). Moreover, the effectiveness of two kinds of trap for collecting scolytids, bottle traps with windows (situated at 1 m and 3 m above ground level) and funnel traps, was tested to control the scolytid wood-boring beetles. Funnel traps collected significantly more individuals than bottle traps. The scolytid diversity collected by each kind of trap was dissimilar, having only 60 % of species in common. These scolytids, especially *Xyleborus* spp., are among the most harmful cacao pests in America, because of their mutualistic association with *Ceratocystis cacaofunesta* (Mazón et al. 2013).

Phytosanitary Risk

Because most forms of the species are easily transmitted in cuttings, unrestricted movement of cuttings or other propagative material is potentially dangerous. It is likely that *Ceratocystis* has been spread to new countries or regions through cuttings of *Populus*, *Theobroma*, *Eucalyptus*, and *Syngonium* and on storage roots of *Ipomoea*. Circumstantial evidence points to packing materials as the source of the plane tree pathogen in southern Europe. *Ceratocystis* is known to survive up to 5 years in wood, probably in aleurioconidia form. *Ceratocystis fimbriata* is listed among the highest risk pathogens that could be imported into the United States on eucalyptus logs and chips from South America (Kliejunas et al. 2001). The

Platanus form (*C. fimbriata* f. *platani*) is listed as an EPPO A2 quarantine pest (OEPP/EPPO 1986).

12.7 Summary

The genus *Ceratocystis sensu stricto* Ellis & Halst. includes many aggressive plant pathogens causing wilt, canker stain diseases, and tissue rot on a wide variety of perennial and economically important agronomic crop worldwide.

In recent years, the recognition of the economic importance of *Ceratocystis* has resulted in an intense scientific interest, as evidenced by an increase in publications.

The major threat to agriculture from *Ceratocystis* is the emergence of new fungal species and their appearance on new hosts and in new geographical areas.

Ceratocystis wilt of cacao is reemerging as an important disease and represents a serious threat to major cacao producers in the world.

The taxonomic history of the genus *Ceratocystis* is complex and has been intertwined with several other genera like *Ophiostoma*, *Leptographium*, and *Raffaelea*. Consequently, the genus has been subjected to many name changes.

The genus *Ceratocystis fimbriata* was placed into the order Microascales, family Ceratocystidaceae. This delimitation has made it possible to study the genus independently and discover new cryptic species.

The genus *Ceratocystis* accommodates many important fungi that include many plant pathogens. Recent studies showed high genetic diversity in this group and have resulted in the proposal to establish species complexes within the genus.

The traditional biological species concepts is inappropriate for this group with high rates of asexual reproduction and several morphological similarities; the similarity in these group can be explained by convergent evolution linked to an association with the dispersal insects.

Species in *Ceratocystis* has been recently defined based on unique morphological characteristics, by mating studies, intersterility tests, host ranges, and phylogenetic inference base on DNA sequence data. However, additional research and continued investigation are required to clearly define all of the species within this genus.

The causal agent of Ceratocystis wilt of cacao is a newly described species, *Ceratocystis cacaofunesta* (formerly *C. fimbriata*), that causes a serious disease of *Theobroma cacao* in Central and South America.

Ceratocystis spp. exhibits the three reproductive modes described in filamentous ascomycetes: vegetative propagation, sexual, and asexual reproduction.

C. cacaofunesta is homothallic by a unidirectional mating-type switching mechanism.

The classical symptoms of the Ceratocystis wilt of cacao are chlorosis of the leaves and wilt within 2–4 weeks after the infection. Leaves remain attached to the plant for weeks even after the death of the plant.

The cacao disease responses, both resistant and susceptible, to *C. cacaofunesta* infection included (1) discoloration of primary walls of infected xylem vessels and

the surrounding parenchyma cells; (2) mobilization of polyphenolic in parenchyma cells; (3) translocation and accumulation of starch in the xylem; and (4) production of gums and tyloses.

Wilt symptoms of cacao infected by *C. cacaofunesta* could be due to toxin activity combined with vessel occlusion.

Ceratocystis species maintain low levels of genetic diversity within populations, because the main reproduction method is asexual and its current dispersal mechanism is highly associated with human transport of clonal isolates.

A strong association with bark beetles for dispersal was reported in many *Ceratocystis* species. It is *currently c*onsidered an inefficient disperser due to the large-scale human-related migrations; however, the past evolution of *Ceratocystis* populations and species might be *highly shape*d by this ecological association. Further studies are necessary to establish the current role of bark beetles and *Ceratocystis* infections.

Wound avoidance is the key to the management of *Ceratocystis* spp. diseases.

The eradication of Ceratocystis wilt has been possible by following good sanitation practices when epidemics caused by *Ceratocystis* are local.

Clean nursery stocks could be produced if care is taken to make the collections of cuttings from trees free of diseases. Follow up monitoring of nurseries stocks should be done using molecular tools.

Treating trees with fungicides to prevent Ceratocystis wilt can be problematic because of the difficulty of treating internal vascular tissues and in providing a single sustained dose.

In cacao, resistant genotypes are the most efficient strategy against *C. cacaofunesta,* because it is more stable and beneficial to the environment.

Because Ceratocystis wilt of cacao kills the plant, fungicides have the potential to prevent crop losses, but in most cases are not economically feasible.

Biological control has the potential to control against Ceratocystis wilt of cacao, but requires field validation.

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