CHAPTER 1

The genus *Ceratocystis* with special reference to species with hat-shaped ascospores occurring on forest trees

1. INTRODUCTION

Globally, commercial forestry plantations cover about 30 % or up to 3,934,890,000 ha of land (Anonymous 2003). The trees most extensively planted are softwoods, eucalypt-, and wattle-trees (Evans 1982, Kanowski 1997, Anonymous 2003). Trees from these planting programmes are used for structural timber, pulp and paper production, utility poles, fuel and mine props (Sutton 1995, Anonymous 2003).

Diseases of forest trees are known to have a substantial influence on fibre production resulting not only in tree death, but greatly influencing quality, growth and yield. In commercial plantation forestry, *Ceratocystis* Ellis & Halst. spp. were first reported early in the 1900's (Halsted 1890, Hedgcock 1906). The first *Ceratocystis* sp. to be reported on a forest tree, *Liquidambar styraciflua* L., was *C. moniliformis* Hedgcock (Hedgcock 1906). *Ceratocystis polonica* (Siemaszko) Moreau and *C. laricicola* Redfern & Minter are two species that cause substantial damage to conifers (spruce) (*Picea* spp.) and Larch (*Larix deciduas* Mill.) (Redfern *et al.* 1987, Christiansen & Solheim 1990). These fungi are associated with the bark beetles *Ips typographus* L. (Coleoptera: Scolytidae) and *Ips cembrae* Heer (Coleoptera: Scolytidae), respectively.

The genus *Ceratocystis* resides in the phylum Ascomycota Whittaker (Hunt 1956), and in the order Microascales (Luttrell) Malloch (1970). This order includes genera with long necked ascomata and sticky ascospore masses adapted to insect dispersal (Hunt 1956, Upadhyay 1981). The ascospores are mostly dextrinoid (yellowish brown or reddish brown in colour) when young, without germ pores. The thin walled asci are evanescent and produce eight ascospores that are often formed in chains (Benny & Kimbrough 1980, Kirk *et al.* 2001). *Ceratocystis* further groups within the class Sordariomycetes (Eriksson & Winka 1997). This class of fungi is characterised by the production of perithecioid ascomata, and the presence of unitunicate asci, often with apical annuli (Kirk *et al.* 2001). Species of *Ceratocystis* are generally treated in their own family, Ceratocystidaceae Locq., which is characterised by the absence of stromata and dark, usually long necked ascomatal perithecia with ostiolar hyphae (Kirk *et al.* 2001).

The genus *Ceratocystis* includes several economically important plant pathogens of trees, agricultural crops and herbaceous plants (Alexopoulos 1962, Seifert, Wingfield & Kendrick 1993). Collectively, species in this genus infect a wide variety of commercially

important food crops (e.g. pineapple, banana, sweet potato, sugarcane, nut and stone fruits, beans, mangoes, dates, coffee and cacao), agronomic crops (e.g. cotton, tobacco, rubber), timber (e.g. eucalypt-, wattle- and plane trees), as well as amenity species (e.g. plane, myrtle beech, sugar maple, aspen, white beach) (Grylls & Seifert 1993, Kile 1993). Species of *Ceratocystis* are responsible for a wide range of disease symptoms including wood stain, canker diseases, vascular wilts and root diseases. There are also a few species to which no particular economic importance has been attributed (Hunt 1956, Seifert *et al.* 1993).

Until recently, *Ceratocystis* spp. have not been considered a problem in commercial hardwood plantations. However, in the 1980's, *C. fimbriata* Ellis & Halst., was reported as the causal agent of canker and death of *Acacia decurrens* de Wild. plantations in Brazil (Ribeiro *et al.* 1985). Shortly after this, *C. albofundus* De Beer, Wingfield & Morris, was described as the causal agent of wilt and death of *Acacia mearnsii* de Wild. in South Africa (Morris, Wingfield & De Beer 1993, Wingfield *et al.* 1996). In 1999, *C. fimbriata* was reported to cause rapid wilt and death of *Eucalyptus* spp. in the Republic of Congo and Brazil (Laia, Alfenas & Harrington 1999, Roux *et al.* 2000). The same fungus was also reported killing *Eucalyptus* trees in Uganda (Roux, Wingfield & Byabashaija 2001) and more recently in Uruguay (Barnes *et al.* 2003a). Four other *Ceratocystis* spp. are known to occur on *Eucalyptus* trees. These include *C. moniliformis* (Hedgcock 1906), *C. eucalypti* Yuan & Kile (Kile *et al.* 1996), *C. moniliformopsis* Yuan & Mohamm. (Yuan & Mohammed 2002) and *C. pirilliformis* Barnes & Wingfield (Barnes *et al.* 2003b). The former three are not considered to be pathogens, while *C. pirilliformis* has not been tested for its pathogenicity.

Species of *Ceratocystis* have a worldwide distribution and are being reported from an increasing number of hosts and geographical areas. The reports of *C. fimbriata* from *Eucalyptus* spp. and *C. albofundus* from *Acacia* have, led to increased concern regarding their threat to commercial forestry, especially in the tropics and the Southern Hemisphere (Roux *et al.* 2003). The aim of this review is to summarize the taxonomic history of *Ceratocystis* and to provide an overview of knowledge pertaining to *Ceratocystis* spp. with hat-shaped ascospores, associated with forest trees.

2. TAXONOMIC HISTORY

The history of *Ceratocystis* dates back more than a century. The genus was first established in 1890, in association with black rot of sweet potato (*Ipomoea batatas* Lam.) in New Jersey, USA (Halsted 1890). The causative fungus was named *C. fimbriata,* which is also the type species of this genus (Halsted 1890, Halsted & Fairchild 1891). The first description of *C. fimbriata* was, however, inaccurate, as Ellis and Halsted mistook the ascospores for conidia, as they failed to observe asci. In 1892 Saccardo, unaware of the oversight of Ellis & Halsted, transferred *C. fimbriata* to *Sphaeronaema fimbriatum* Fr. (Saccordo 1892). Von Hönel (1918) divided the genus *Ceratostomella* Sacc., which was established for fungal species with colourless ascospores by Saccardo (1878), into two groups based on morphology. The one group, residing in *Ceratostomella*, represented species with fleshy perithecia and cylindrical asci. The other group was provided with the name *Linostoma* Hönel and consisted of species with carbonaceous perithecia with long necks and ovoid asci containing spores arranged in several rows (Von Hönel 1918). *Sphaeronaema fimbriatum* was transferred to *Linostoma* (Von Hönel 1918). Unfortunately the name *Linostoma* had been assigned to a genus of flowering plants in the *Thymeleacaeae* Juss. Von Sydow and Sydow (1919), therefore, established the genus *Ophiostoma* Syd. & Syd. and relegated all the species previously in *Linostoma* to this newly established genus, separated from the genus *Ceratostomella.*

Almost thirty years after *Ceratocystis* was first discovered, Elliott (1923) found that the presumed pycnidia of the fungus were in fact perithecia with ascospores emerging from deliquescent asci. He thus transferred *S. fimbriatum* to *Ceratostomella* Sacc. (Elliot 1923). The work of both Von Hönel (1918) and Von Sydow & Sydow (1919) was, however, not accepted until Melin and Nannfeldt (1934) reduced the genus *Endoconidiophora* Münch, which was established for species that formed conidia endogenously, to *Ceratostomella.* They transferred *Ceratostomella fimbriata*, *C. paradoxa* (Dade) C. Moreau and *C. adiposa* Butl. to *Ophiostoma*, due to the fact that they had *Chalara* (Corda) Rabenh. conidial stages (Melin & Nannfeldt 1934). Melin & Nannfeldt (1934) stated that the oldest genus name *Endoconidiophora* should be used, but this name would be confusing and thus suggested the genus name *Ophiostoma* until such a time that a more suitable name could be suggested. Melin and Nannfeldt (1934) divided *Ophiostoma* into two sections, *Brevirostrata* Nannfeldt and *Longirostrata* Nannfeldt. Short, conical perithecial necks characterised the section *Brevirostrata*, while longer filiform necks characterised

Longirostrata. The latter was further subdivided into two groups, those with endogenous *Chalara*-type conidia, and those with exogenous conidia.

In 1950, some species of the genus *Endoconidiophora* were transferred back to *Ceratocystis* (Bakshi 1950). Bakshi (1951) published a significant paper separating *Ceratocystis* and *Ophiostoma*. Although Bakshi's (1951) division of *Ceratocystis* and *Ophiostoma* was generally accepted (Moreau 1952, Hunt 1956), some authors chose to treat the two genera as one (Von Arx 1952, Von Arx & Műller 1954, Upadhyay 1981). This resulted in considerable confusion in later taxonomic classification of species in these genera.

Distinct differences between the anamorphs of *Ceratocystis* and *Ophiostoma* have been observed (Moreau 1952, Hunt 1956, Nag Raj & Kendrick 1975, Upadhyay 1981). Hunt (1956), separated *Ceratocystis* into two groups based on anamorph characteristics. The first group consisted of species, that produced conidia endogenously while the other group had conidia developing both endogenously and exogenously. The second group was then subdivided into two categories, those with mycelial conidia and those with *Graphium* Corda or *Leptographium* Lagerb. & Melin conidial types.

Olchowecki & Reid (1974), recognized four groups in *Ceratocystis* based on ascospore morphology. These were referred to as the Minuta, Ips, Fimbriata and Pilifera groups. Upadhyay and Kendrick (1975) accepted *Ceratocystis* but established the new genus, *Ceratocystiopsis*, to accommodate species with falcate generally aseptate (1-septate in one species), ascospores with hyaline, gelatinous sheaths with attenuated ends, and asci fusiform or clavate. The Minuta group of Olchowecki & Reid (1974) was more or less equivalent to the genus *Ceratocystiopsis* Upadhyay & Kendr. (Upadhyay & Kendrick 1975, Harrington 1981).

It is accepted today that species of *Ceratocystis* have *Thielaviopsis* Went (*Chalara*) (Paulin-Mahady, Harrington & McNew 2002) anamorphs with enteroblastic conidiogenesis, while species of *Ophiostoma* have anamorphs in a number of genera, most commonly *Sporothrix* Hektoen & Perkins, *Leptographium*, *Pesotum* Crane and *Hyalorhinocladiella* Upadhyay & Kendr., with holoblastic conidiogenesis (Von Arx 1974, De Hoog & Scheffer 1984, Samuels 1993, Wingfield, Viljoen & Wingfield 1999,

Upadhyay 1981). Species of *Ceratocystiopsis* have anamorphs that are similar to those of *Ophiostoma* (Wingfield 1993).

The three genera, *Ceratocystis*, *Ophiostoma* and *Ceratocystiopsis* have collectively been known as *Ceratocystis sensu lato* (De Hoog & Scheffer 1984, Wolfaardt, Wingfield & Kendrick 1992, Wingfield *et al.* 1994). Species of *Ceratocystis* are very different to those of *Ceratocystiopsis* and *Ophiostoma* based on morphology, physiology and molecular data (Smith, Patik & Rosinski 1967, Weijman & De Hoog 1975, Harrington 1981, De Hoog & Scheffer 1984, Hausner, Reid & Klassen 1993a). *Ceratocystiopsis* and *Ophiostoma*, however, often share characteristics (De Hoog & Scheffer 1984, Hausner *et al.* 1993a, Wingfield 1993). Due to the close relationship and similarities of *Ceratocystiopsis* and *Ophiostoma*, various authors have proposed their synonymy (Wingfield, Van Wyk & Marasas 1988, Hausner *et al.* 1993a,b, Wingfield 1993), which is now widely accepted.

Ceratocystis and *Ophiostoma* can also be separated at the ultrastructural level. In-depth studies of the development of teleomorph structures revealed that young asci in *Ceratocystis* line the periphery of the inner perithecium while in *Ophiostoma*, the young asci are produced from the base of the inner perithecium (Van Wyk, Wingfield & Van Wyk 1991). The centrums of *Ceratocystis* and *Ophiostoma* also differ with regard to the type of cells present. *Ceratocystis* spp. have filamentous cells while *Ophiostoma* spp. have pseudoparenchymatous cells (Benny & Kimbrough 1980).

Biochemical studies on *Ceratocystis* and *Ophiostoma* have clearly shown that they should reside in separate genera. *Ceratocystis* spp. are sensitive to the antibiotic cycloheximide while *Ophiostoma* spp. are highly tolerant (Harrington 1981). The composition of the cell walls of *Ceratocystis* and *Ophiostoma* are also different. *Ophiostoma* spp. contain cellulose and rhamnose in their cell walls while *Ceratocystis* spp. contain neither of these compounds (Smith *et al.* 1967, Jewell 1974, Weijman & De Hoog 1975). *Ceratocystis* spp. are thus more like other ascomycetous fungi with chitin in their cell walls.

Contention regarding the separation between *Ceratocystis* and *Ophiostoma* has been resolved largely in the last decade, based on DNA sequence analysis. The two genera form distinct clades when their DNA sequences are compared (Hausner *et al.* 1992, 1993a,c, Spatafora & Blackwell 1994, Wingfield *et al.* 1994, Witthuhn *et al.* 1998, 1999). Some of the genes used to separate these two fungal groups initially have been the large sub-unit

(LSU) ribosomal RNA (rRNA) (Hausner *et al.* 1993a,b, Wingfield *et al.* 1994) and the small sub-unit (SS) rRNA (SSrRNA) (Hausner *et al.* 1992, 1993a,b). The separation of *Ophiostoma* and *Ceratocystis* is now widely accepted. The two genera are clearly phylogenetically distinct from each other. Phylogenetically, *Ceratocystis* resides in the Microascales and *Ophiostoma* in the Ophiostomatales (Hausner *et al.* 1993a,c, Wingfield *et al.* 1996, Witthuhn *et al.* 1998, 1999).

3. MORPHOLOGY

In the early days of fungal taxonomy, morphological characteristics represented the primary means to distinguish between mycelial fungi. Consequently, many oversights emerged and these have led to considerable taxonomic confusion. In this regard, species in *Ceratocystis* have been no exception. As mentioned earlier in this review, the ascospores were mistakenly identified as conidia because the rapidly deliquescing asci were not seen (Halsted 1890). Twenty-five years later, it was observed that these "conidia" were in fact ascospores and the fungus was placed in the genus thought to be correct at that time (Elliott 1923).

3.1 Teleomorph

In *Ceratocystis*, the teleomorph state provides the primary morphological characteristics to distinguish between most species. Important characteristics include the shape, size, colour and ornamentation of the ascocarp bases, the attachment of the neck to the base, the length and ornamentation of the neck and the shape and size of the ascospores.

The ascomatal bases of *Ceratocystis* spp. are enlarged and have a characteristic bulbous form, which is either globose e.g. *C. moniliformis* (Hedgcock 1906), globose but flattened or flask-shaped, e.g. *C. fimbriata* (Upadhyay 1981) or pear shaped, e.g. *C. pirilliformis* (Barnes *et al.* 2003b). The bases can be ornamented or unornamented. When ornamented, it is in the form of short conical spines, which can be brown to black, thin or thick-walled, or they can be in the form of septate, unbranched hyphal hairs (Hunt 1956, Upadhyay 1981). *Ceratocystis moniliformis* (Hedgcock 1906) and *C. moniliformopsis* (Yuan & Mohammed 2002) are the only species with perithecial bases that are covered with short conical spines. Almost all *Ceratocystis* spp. have pale brown to black bases, with the exception of *C. albofundus*, that has hyaline bases (Morris *et al.* 1993, Wingfield *et al.* 1996).

The ascomatal necks of *Ceratocystis* spp. are dark brown to black, except for the apices that are pale brown to hyaline. This characteristic is, therefore, not useful for identification. The length of the necks can, however, be used as a means for discrimination between species. *Ceratocystis moniliformis*, for example, has necks of 500–900 µm long (Hedgcock 1906), while *C. fagacearum* (Bretz) Hunt (Upadhyay 1981) and *C. moniliformopsis* (Yuan & Mohammed 2002) have necks of 265–500 µm and 480–780 µm in length, respectively. The tips of the necks of most *Ceratocystis* spp. are ornamented with hyaline to pale brown ostiolar hyphae. The orientation of the ostiolar hyphae at the apices of the necks can also be used for diagnostic purposes. Some species, for example *C. albofundus* (Wingfield *et al.* 1996) have convergent ostiolar hyphae, while others have divergent ostiolar hyphae such as those found in *C. moniliformis* (Hedgcock 1906). The bases of the necks are also useful in distinguishing between species of *Ceratocystis*, due to a small number of species that have a disc-shaped attachment to the bases of the ascomata. These disc-shaped attachments are found in *C. moniliformis* (Davidson 1935) and *C. moniliformopsis* (Yuan & Mohammed 2002).

The time of extrusion of the ascospore masses in *Ceratocystis* varies between species as well as temperature. Three days after a spore drop has been inoculated onto artificial agar and incubated, *C. moniliformis* already produces mature ascomata with ascospores (Hedgcock 1906). *Ceratocystis fimbriata*, for example, only produces ascomata with ascospores after 7 days of incubation at 25 °C (Hunt 1956).

Ceratocystis spp. have diverse ascospore morphologies. Some are hat-shaped in side-view due to flanged appendages such as in the case of *C. moniliformis* (Hedgcock 1906) and *C. pirilliformis* (Barnes *et al.* 2003b). *Ceratocystis autographa* Bakshi was described as having oblong-ellipsoidal ascospores (Bakshi 1951, Grylls & Seifert 1993) although this species has an unclear taxonomic description. *Ceratocystis stenospora* Griffin was described with fusiform to falcate ascospores (Griffin 1968, Grylls & Seifert 1993) but the validity of this species is also not certain. All the ascospores in these species are hyaline in colour, one-celled, smooth, sheathed and produced in a gelatinous matrix (Hunt 1956, De Hoog 1974, Van Wyk *et al.* 1991).

3.2 Anamorph

The anamorphs of *Ceratocystis* spp. have traditionally been accommodated in *Chalara* (Von Arx 1974, Harrington 2000). A recent phylogenetic study by Paulin-Mahady *et al.* (2002) showed that the anamorphs of these fungi should most appropriately be treated in *Thielaviopsis*. Their study, based on DNA sequence data, also emphasised the fact that, aside from *C. moniliformis* and *C. fagacearum*, most anamorph species of *Ceratocystis* produce thick-walled aleuroconidia from the tips of specialized hyphae. The genus *Thielaviopsis* is based on *T. ethacetia* Went (Nag Raj & Kendrick 1975), which was later synonymized with *T. paradoxa* (de Seynes) Höhn, the anamorph of *C. paradoxa* (Dade) Moreau. *Chalaropsis* Pyronel., which was characterised as an endogenous and an enteroblastic conidial phase was reduced to synonymy with *Thielaviopsis* (Nag Raj & Kendrick 1975).

Thielaviopsis spp. are characterised by the formation of aleuroconidia on specialised hyphae (Paulin-Mahady *et al.* 2002). These aleuroconidia are thick walled and are usually dark in colour. They can be formed in chains or can occur singly. Aleuroconidia are not a prerequisite for species to be placed into this genus (Paulin-Mahady *et al.* 2002). Phialides produce cylindrical conidia through ring wall building, which can be extruded in chains or singly. The conidia may remain hyaline or become thick-walled and dark with maturity (Nag Raj & Kendrick 1975, Minter, Kirk & Sutton 1982, 1983, Nag Raj & Kendrick 1993). When known, teleomorphs of *Thielaviopsis* most appropriately reside in the genus *Ceratocystis* (Paulin-Mahady *et al.* 2002).

A distinguishing characteristic of the anamorphs of *Ceratocystis*, is the presence or absence of aleuroconidia, also known as chlamydospores. These are known to enable species to survive in soil. For example, *C. fimbriata,* which is known to be able to survive in soil for many years, has chlamydospores (Rossetto & Riberio 1990, Marin *et al.* 2003), while *C. moniliformis*, which is not known to survive is soil does not produce chlamydospores (Hedgcock 1906, Upadhyay 1981). The size of these structures is also taxonomically useful e.g. *C. fimbriata* being 9–15 x 7.5–11 µm (Upadhyay 1981), while those in *C. pirilliformis* are 8–12 x 5.5–8 µm (Barnes *et al.* 2003b).

The size of *Thielaviopsis* conidia can be used to distinguish between species of *Ceratocystis*. For example, the length of the cylindrical conidia in *C. fimbriata* ranges from 9–21 µm (Upadhyay 1981), while those of *C. moniliformis* are 6–8 µm (Hedgcock

1906). The barrel-shaped conidia in *C. fimbriata* are 9–14 x 5–15 µm in diameter (Upadhyay 1981), while those of *C. pirilliformis* are 4–6 x 3–4 µm (Barnes *et al.* 2003b).

The size of the phialidic conidiogenesis cells differs between species of *Ceratocystis* and can be used to distinguish between some species. *Ceratocystis fimbriata*, for example has phialides that are 35-130 x 2.5-6 µm, at the base (Upadhyay 1981), while those of *C. moniliformopsis* range between 5-32.5 x 4-5 µm, at the base (Yuan & Mohammed 2002).

4. PHYLOGENETIC PLACEMENT

The use of DNA sequence data has revolutionized the taxonomic placement of fungal species. Phylogenetic relationships between distantly related taxa, such as genera of fungi, can be inferred from slowly evolving gene regions, for example, the LSU rRNA gene. In contrast closely related taxa such as species can be distinguished by using rapidly evolving gene regions (e.g. Internal Transcribed Spacer regions {ITS}) (Bruns, White & Taylor 1991, Messner 1995, Mitchell, Roberts & Moss 1995). The rRNA genes consist of highly conserved regions interspersed with variable regions and can, therefore, be used to study the relatedness between both distantly and closely related species (Jorgenson & Cluster 1988, Taylor *et al.* 1993). DNA sequence data from rRNA genes have been used effectively to determine the phylogenetic relationships among ophiostomatoid fungi and have contributed significantly to understand the differentiation between *Ophiostoma* and *Ceratocystis* (Hausner *et al.* 1992, 1993a,b,c, Spatafora & Blackwell 1994, Wingfield *et al.* 1994).

The genes used to distinguish between *Ophiostoma* and *Ceratocystis* have been used to show that *Ceratocystis* spp. group within the Microascales, while *Ophiostoma* spp. reside in the Ophiostomatales (Spatafora & Blackwell 1994). Partial rDNA sequence data have also contributed to reducing *Ceratocystiopsis* spp. to synonymy with *Ophiostoma* (Hausner *et al.* 1993c). It has thus been with the aid of DNA sequence data, that *Ophiostoma* and *Ceratocystis* have been unequivocally separated and shown to be phylogenetically distantly related.

Various gene regions have been used to study the taxonomic placement and relationships among species of *Ceratocystis*. The ITS region of the rRNA genes were used by Witthuhn *et al.* (1999) to show that the genus can be divided into two discrete clades. One of these

clades is more commonly known as the *C. coerulescens* clade, while the other is referred to as the *C. fimbriata* clade. The ITS region has also been used to distinguish between *Ceratocystis* spp. that are morphologically similar and thought to be the same species. It was proved by DNA sequence data, from the ITS regions, that there were in fact five distinct species within the *C. coerulescens* group (Witthuhn *et al.* 1998). *Ceratocystis laricicola* and *C. polonica* are morphologically difficult to distinguish and are often identified based only on their different hosts and insect associations (Yamaoka *et al.* 1997). Comparison of ITS sequences for these two species has, however, not supported the notion that they are distinct species (Harrington & Wingfield 1998, Witthuhn *et al.* 1998). Other genes that are frequently used to distinguish between species are; MAT genes (Witthuhn *et al.* 2000, Marin *et al.* 2003) and β-tubulin genes (Loppnau & Breuil 2003).

Various molecular markers, such as Restriction Fragment Length Polymorphisms (RFLPs) and microsatellites have been used in the identification of *Ceratocystis* spp. and in studies pertaining to populations of these species. RFLPs have been used to distinguish between closely related *Ceratocystis* spp. such as *C. coerulescens*, *C. pinicola* Harrington & Wingfield, *C. douglassi* (Davidson) Wingfield & Harrington, *C. resinifera* Harrington & Wingfield, *C. rufinipenni* Wingfield Harrington & Solheim, *C. polonica* and *C. adiposa* (Witthuhn *et al.* 1999). This technique uses the principle that nucleotides at a given place in the DNA of different species might differ, and thus, the restriction sites might be different. As a result, different sized fragments are seen on an agarose gel if there are differences in the nucleotide sequence. When a PCR-RFLP was done on the β-tubulin gene DNA, *Ceratocystis* spp. causing sapstain of conifer trees showed different bands, resulting in a rapid method whereby species can be distinguished (Loppnau & Breuil 2003).

Simple Sequence Repeats (SSRs) or microsatellites are more specific than RFLP's and can detect up to five times more variation (Tautz 1989). Microsatellites are typically used to study populations rather than single species, as they have a high level of polymorphism and can distinguish between individuals of the same species. They are co-dominant markers, compared to the dominant RFLP markers. Microsatellites consist of runs of short repeating sequences that can be variable in number between individuals (Jarne & Lagoda 1996) and can also identify different alleles within a single locus in individuals. By designing DNA primers flanking these repeat regions, the polymorphisms between

individuals can be studied easily as the microsatellites are inherited in a Mendelian fashion (Schlötterer & Pemberton 1988, Tautz 1989). Microsatellites have been used in a study of *C. fimbriata* to reflect intra-specific relationships between isolates and to compare the results of the population comparison with those emerging from phylogenies based on DNA sequencing (Barnes *et al.* 2001). The microsatellite markers developed for *C. fimbriata* have also been used for *C. albofundus* (Barnes 2002, Nakabonge 2002), which also provides an indication that microsatellite markers can sometimes be used to study population structures in closely related species.

5. BIOCHEMISTRY

The biochemistry of fungi plays an important role in the expression of its morphology and development. As early as 1921, biochemistry was used to differentiate between fungi. Von Wettstein (1921) divided the fungal Kingdom into two groups based on the carbohydrate constitution of the cell walls. The one group of fungi contained cellulose while the other group, including the ascomycetes contained chitin in their cells. In terms of the *Ophiostomatoid* fungi, the presence or absence of cellulose and chitin, as well as rhamnose in their cell walls has been used to distinguish *Ceratocystis* spp. from *Ophiostoma* spp. (Smith *et al.* 1967, Jewel 1974).

Most *Ceratocystis* spp. are deficient in their ability to produce vitamins such as thiamine, pyridoxine, and biotin (Robbins & Ma 1942). These vitamins can be added to artificial media to induce growth. Thiamine, as well as calcium, enhance the production of sexual structures, and thus facilitate sexual reproduction of these fungi (Robbins & Ma 1942, Hawker 1966, Upadhyay 1981).

Species of *Ceratocystis* produce intense fruity odours in young cultures, due to the production of monoterpenes (Hunt 1956, Lanza & Palmer 1977, Hanssen 1993). Monoterpenes are produced only when the nitrogen source is very low (Lanza & Palmer 1977). Odours tend to differ when different media are used to culture these fungi (Lanza, Ko & Palmer 1976, Lanza & Palmer 1977). The fruity aroma produced by these fungi is important in attracting insects that facilitate their dispersal.

Species of *Ceratocystis* can be placed in two groups based on the production of fruity aromas. The one group consists of species that do not produce the fruity aromas, for

example, *C. polonica* (Upadhyay 1981, Yamaoka *et al.* 1997). These fungi tend to have very specific insect vectors such as bark beetles (Yamaoka *et al.* 1997). The other group consists of species, which produce strong fruity odours to attract a wide variety of insects, for example *C. fimbriata* (Upadhyay 1981). The latter group have loose associations with insects such as flies (Diptera) and picnic beetles (Coleoptera: Nitidulidae).

6. REPRODUCTION

In general, ascomycetous haploid fungi have three basic reproductive strategies. They can either reproduce vegetatively, asexually or sexually (Alexopoulos 1962). When a fungus reproduces vegetatively, an unspecialised part of the original culture becomes detached from the main body of mycelium to form independent individuals. In asexual reproduction, specialized reproductive bodies are formed from which the new individuals are released, independent of nuclear fusion. Both of these forms of reproduction lead to progeny that are identical to their parents, both phenotypically and genotypically. Sexual reproduction relies on the fusion of two compatible nuclei (n) and a subsequent reduction division follows which produces new haploid individuals (Hawker 1966).

Ceratocystis spp. have a number of reproductive strategies that contribute to differing levels of genetic diversity. Most *Ceratocystis* spp. are self-fertile (homothallic), thus there is no need for two different individuals to reproduce via meiosis. This is found, for example, in *C. fimbriata* and *C. virescens* (Davids.) Moreau (Olson 1949, Webster & Butler 1967a,b, Harrington & McNew 1997). Species of *Ceratocystis* can also be heterothallic, and therefor need two separate individuals with opposite mating structures or genes to reproduce. Some heterothallic species in *Ceratocystis* are: *C. paradoxa*, *C. radicicola* Bliss, *C. fagacearum* and *C. eucalypti* (Heptig, Toole & Boyce 1952, Kile *et al.* 1996).

Sexual compatibility in heterothallic ascomycetes is determined by two alleles (idiomorphs), MAT-1 and MAT-2, at a single mating type locus. Thus, two strains must be of opposite mating types to produce ascospores (Glass & Kuldau 1992). Some *Ceratocystis* spp. show uni-directional mating type switching where self-fertile or self-sterile progeny can be produced from single ascospores, e.g. *C. coerulescens* and *C. albofundus* (Harrington & McNew 1997). MAT-2 strains can switch to MAT-1 and self,

but MAT-1 progeny cannot switch to MAT-2 and therefore remain self-sterile (Harrington & McNew 1997).

7. DISTRIBUTION & HOST RANGE

The genus *Ceratocystis* has a worldwide distribution, and has been reported from all continents except Antarctica (Table 1 & 2) (Kile 1993). *Ceratocystis moniliformis,* for example, has been found in Africa (Luc 1952, Witthuhn *et al.* 1999), North America (Von Schrenk 1903, Davidson 1935), Europe (Bakshi 1951) and Asia (Kitajima 1936, Roldan 1962, Witthuhn *et al.* 1999) (Table 1). *Ceratocystis fimbriata* has also been found in Africa (Roux *et al.* 2000), the Americas (Sharples 1936, Pontis 1951, Riberio *et al.* 1985, Barnes *et al.* 2003a), Asia (Halsted 1890, Kile 1993) and Europe (Kile 1993) (Table 2). There are species of *Ceratocystis*, however, that have a limited distribution such as *C. pirilliformis*, which has been found only in Australia (Barnes *et al.* 2003b), while *C. albofundus* has been found only in Africa (Wingfield *et al.* 1996, Roux *et al.* 2001).

The host range of *Ceratocystis* spp. differs between species and geographical areas. Some *Ceratocystis* spp. such as *C. moniliformis* are found on many plants including fruit trees, forest trees, fruits and crops (Table 1). Others, such as *C. polonica* (Table 3), are strongly associated with a particular host and are carried by a specific insect, in this case *Picea* infested with *Ips typographus* (Yamaoka *et al.* 1997). Certain species are pathogenic to some hosts while non-pathogenic to others. *Ceratocystis fimbriata* is considered as a species with a wide host range. However, an increasing number of studies indicate that this species might represent host specific forms (Leather 1966, Webster & Butler 1967b, Harrington 2000).

8. INFECTION, DISEASE DEVELOPMENT AND SYMPTOMS

Most *Ceratocystis* spp. that infect woody plants require wounds for infection (Seifert *et al.* 1993). On trees, the spores germinate, and the mycelium enters through wounds and moves through the xylem into the ray parenchyma cells. The fungus subsequently causes dark reddish brown to purple discolouration of the tissues. Chlamydospores, the survival structures, produced by some *Ceratocystis* spp., are known to survive in the soil for long periods of time (Moutia & Saumtally 1999). Under optimal conditions, these spores can germinate and infect susceptible crops via the roots (Kile 1993, Moutia & Saumtally 1999). Disease symptoms caused by *Ceratocystis* spp. include fruit rots, sapstain of

timber, vascular wilts, vascular stains, cankers and root and stem rots (Kile 1993, Seifert *et al.* 1993). Some of the more economically important species of *Ceratocystis* are listed in Table 3.

9. DISPERSAL

Ceratocystis spp. employ a variety of dispersal mechanisms. They are dispersed via root grafts, as seen between *Platanus* trees (Skelly & Merril 1968, Accordi 1986), as well as by means of infested soil (Rossetto & Riberio 1990, Moutia & Saumtally 1999, Marin *et al.* 2003). *Ceratocystis fimbriata* has been reported to have the capacity to survive in soil for years (Rossetto & Riberio 1990, Moutia & Saumtally 1999, Marin *et al.* 2003). The fungus can also be dispersed by air, as an indirect association with insects and in the frass that insects produce when they tunnel through the wood, which can be dispersed by wind or water (Iton 1960). There have been no reports on the spread of these fungi from or within seeds (Upadhyay & Kendrick 1975, Harrington 2000). Agricultural practices as well as thinning of trees with the aid of infected machetes or pruning tools also aid in dispersal (Grosclaude, Olivier & Romiti 1995, Marin *et al.* 2003). In Colombia for example, the soil borne *C. fimbriata* commonly infects wounds made by the shoes of farm workers who use planted trees as support when climbing steep hills (Marin *et al.* 2003).

Insect transmission is the main method of dispersal for *Ceratocystis* spp., especially in living trees (Leach 1940, Himelick & Curl 1958, Upadhyay 1981). *Ceratocystis* spp. are well adapted for insect dispersal. The ascospores are collected in slimy masses at the apices of long ascomatal necks, (also described as a stalked spore-drop) (Ingold 1961), that stick to the bodies of insects (Leach, Orr & Christensen 1934, Griffin 1968). Some species of *Ceratocystis* also emit fruity odours that attract insects (Lanza & Palmer 1977). Spores that are ingested by the insects pass unharmed through their intestinal tracts (Leach 1940).

There are two distinct categories of association between *Ceratocystis* spp. and insects. Some species have a casual relationship with insects. These are the species that tend to produce fruity odours that attract many species of flies (Diptera) and sap beetles (Nitidulidae) (Himelick & Curl 1958). Species of *Ceratocystis* that have this form of adaptation include *C. fimbriata*, *C. albofundus*, *C. pirilliformis*, *C. fagacearum* and *C. moniliformis*. Another group of *Ceratocystis* spp. tend to not produce fruity odours. These

species rely on very close relationships with specific insects and particularly bark beetles for their dispersal. Thus, *C. polonica* is specifically carried by the bark beetle, *Ips typographus* that infests spruce (*Picea* spp.) in Europe and Asia (Siemaszko 1938, Christiansen & Solheim 1990), *C. laricicola* is carried by *I. cembrae* that infests larch in Europe and Asia (Redfern *et al.* 1987, Yamaoka *et al.* 1998) and C. *rufipenni* Wingfield, Harrington & Solheim is carried by *Dendroctonus rufipennis* Kirby that infests Engelman spruce (*Picea engelmannii* Parry) in Western North America (Solheim & Safranyik 1997, Wingfield, Harrington & Solheim 1997).

10. *CERATOCYSTIS* SPP. WITH HAT-SHAPED ASCOSPORES THAT ARE ASSOCIATED WITH HARDWOOD TREES IN PLANTATIONS

In recent years there has been increased concern regarding the number of *Ceratocystis* spp. that affect exotic plantation forests. *Ceratocystis* spp. group into two distinct clades, namely the *C. coerulescens* and *C. fimbriata* clades (Witthuhn *et al.*1999). There are three species, *C. fimbriata* (Upadhyay 1981), *C. albofundus* (Morris *et al.* 1993) and *C. pirilliformis* (Barnes *et al.* 2003b) in the *C. fimbriata* clade that have hat-shaped ascospores. Of these, *C. fimbriata* and *C. albofundus* are highly pathogenic and cause considerable losses to commercial forestry plantations (Wingfield *et al.* 1996, Harrington 2000). The steadily increasing number of reports of these and other pathogens associated with commercial plantation trees have caused considerable concern amongst forestry companies over the last 10 years. The following section of this review focusses on *Ceratocystis* spp. with hat-shaped ascospores that have been reported from commercial plantations of hardwood trees (Table 4, 5). These fungi are also related to those treated in the research chapters of this thesis.

10.1 ***Ceratocystis fimbriata*** Ellis & Halsted, Bull. N. J. Agric. Sta. 76: 14. 1890.

≡ *Sphaeronaema fimbriatum* (Ell. & Halst.) Sacc., Syll. Fung. 10: 125. 1892.
≡ *Ceratostomella fimbriata* (Ell. & Halst.) Elliott, Phytopath. 13: 56. 1923.
≡ *Ophiostoma fimbriatum* (Ell.& Halst.) Nannf., Svenska Skogsfor. Tidskr.32: 408. 1934.
≡ *Endoconidiophora fimbriata* (Ell. & Halst.) Davidson, J. Agric. Res. 50: 800. 1935.
= *Rostrella coffeae* Zimmermann, Medded. s'lands plantentuin 37: 24. 1900.
= *Endoconidiophora variospora* Davidson, Mycologia 36: 303. 1944.
= *Ceratocystis variospora* (Davids.) Moreau, Rev. Mycol. (Paris) Suppl. Col.17:22. 1952.

= *Ophiostoma variosporum* (Davids.) von Arx. Antonie van Leeuwenhoek 18: 212. 1952.

Ceratocystis fimbriata was first discovered as the causal agent of black rot of sweet potato in 1890 (Halsted 1890) and it is the type species for the genus *Ceratocystis*. It has globose to flask shaped ascomatal bases without ornamentation. The bases, as well as the necks of the ascomata, are dark in colour and almost black (Halsted & Fairchild 1891, Hunt 1956, Upadhyay 1981). *Ceratocystis fimbriata* produces hat-shaped ascospores and abundant chlamydospores (Hunt 1956, Upadhyay 1981). *Ceratocystis fimbriata* grows slowly, 15 mm in 12 days, with an optimal temperature of 22 °C (Hunt 1956, Upadhyay 1981). It is also characterised by the production of fruity, banana like odours in young cultures (Hunt 1956, Upadhyay 1981). Two types of conidia are produced; one cylindrical and the other more barrel-shaped (Hunt 1956, Upadhyay 1981).

Forest trees that are associated with infection by *C. fimbriata* include *Eucalyptus* spp. (Roux *et al.* 2000), *Populus tremula* L. (Aspen) (Wood & French 1963), *Platanus* spp. (Plane) (Jackson & Sleeth 1935) and *Gymnocladus* spp. (Pontis 1951) (Table 2). *Ceratocystis fimbriata* is pathogenic to many different tree crops and infects through wounds (Kile 1993). The symptoms usually seen after infection of *C. fimbriata* on forestry trees range from cankers to wilt and dieback (Pontis 1951, Roux *et al.* 2000). *Ceratocystis fimbriata* has "races" or "strains" that are host specific (Webster & Butler 1967b, Wellman 1972, Harrington 2000). This host specificity seems to correlate with geographic regions (Webster & Butler 1967b, Harrington 2000, Barnes *et al.* 2001, Baker *et al.* 2003).

There has been considerable debate regarding the origin of *C. fimbriata*. It was originally thought to have originated in South America and to have spread on infected plant material to the rest of the world. These hypotheses were, however, based on host specialization, range and DNA sequence data. Recently, Barnes *et al.* (2001) developed microsatellite markers for *C. fimbriata*. These authors compared three populations of *C. fimbriata*; a population from Congo and Uruguay from commercial *Eucalyptus* plantations and a population from Colombia from coffee trees. The study showed a high genetic differentiation between these three populations with minimal gene flow. There was little evidence of recombination within the populations. The population study done by Baker & Harrington (2000), which used microsatellites, nuclear DNA fingerprints & mitochondrial DNA RFLP's concluded that *C. fimbriata* f. *platani* is native to North America.

10.2 ***Ceratocystis pirilliformis*** Barnes & Wingfield, Mycologia 95: 865-871. 2003.

Ceratocystis pirilliformis is a recently discovered species that infects *Eucalyptus* trees in Australia (Barnes *et al.* 2003b). It is phylogenetically closely related to *C. fimbriata* but it has pear-shaped ascomatal bases with thick necks, producing hat-shaped ascospores. The anamorph is typical of *Thielaviopsis* producing both cylindrical and barrel-shaped conidia. It is a slow growing fungus, growing 22 mm in 12 days at an optimal temperature of 25 °C. The cultures appear white at first, becoming grey to olive green (Barnes *et al.* 2003b). *Ceratocystis pirilliformis* produces an aroma similar to that of *C. fimbriata*, which is fruit-like and resembling banana like when cultures are actively growing.

Ceratocystis pirilliformis was isolated from artificial wounds on *Eucalyptus nitens* Dean et Maiden. It causes a sapstain that discolours the wood and may decrease timber value (Barnes *et al.* 2003b). No other reports of this fungus are known and its possible role in disease development must still be determined.

10.3 ***Ceratocystis albofundus*** De Beer, Wingfield & Morris Syst. Appl. Microb. 19: 191-202. 1996.

= *Ceratocystis fimbriata* (Ell. & Halst.) Elliot, Gorter, Morris *Plant Path.* 42 : 814-817. 1993.

Ceratocystis albofundus has hat-shaped ascospores and is the second species to be described as a pathogen of commercial plantation forest trees. It is characterised by light ascomatal bases compared to the dark bases of *C. fimbriata* and *C. pirilliformis* (Morris *et al.* 1993). The ostiolar hyphae of *C. albofundus* are convergent compared to the divergent ostiolar hyphae of *C. fimbriata* and *C. pirilliformis*. Colonies of *C. albofundus* are light in colour and have an optimum growth temperature of 30 °C. *Ceratocystis albofundus* has a typical *Thielaviopsis* anamorph producing both cylindrical and barrel-shaped conidia (Morris *et al.* 1993, Wingfield *et al.* 1996). A sweet smelling aroma, similar to that of *C. fimbriata*, is produced by *C. albofundus*.

Ceratocystis albofundus was first identified in 1977 as *C. fimbriata*, occurring on *Protea* spp. (Gorter 1977). Thereafter, it was reported as the cause of wilt and dieback of a few

Acacia spp. (Morris *et al.* 1993, Wingfield *et al.* 1996). The species name was corrected by Wingfield *et al.* (1996) who recognised it as a new species *C. albofundus*. This fungus is geographically restricted and has been reported only from the African continent.

Ceratocystis wilt caused by *C. albofundus* is characterised by the formation of cankers and lesions on the bark of affected trees and the exudation of gum from the lesions. Internally, the affected trees show signs of discolouration. The result of infection by this fungus is a rapid wilt and dieback of trees, often leading to death within a few weeks after infection (Morris *et al.* 1993).

It has been hypothesised that *C. albofundus* is native to South Africa. This is based on the fact that the fungus is known only from South Africa and occurs on native *Protea* spp. (Roux *et al.* 2001). It has been supported by a population genetic study using the oligonucleotide marker CAT5 as well as restriction digests of the mitochondrial DNA. Recently, it has been shown that microsatellite markers developed for *C. fimbriata* also function for studies on *C. albofundus* (Barnes 2002). Nakabonge (2002), therefore, compared the South African population used by Roux *et al.* (2001) with a Ugandan population of the fungus. They found that the Ugandan population was slightly more diverse than the South African population with very little gene flow detected. *Ceratocystis albofundus* is probably native to Africa and not only to South Africa as has previously been suggested (Nakabonge 2002). *Ceratocystis albofundus* is of great quarantine importance, as it does not occur outside Africa and for example might threaten Australian *Acacia* spp., both in countries where they are grown as exotics or in their native environment.

10.4 ***Ceratocystis moniliformis*** (Hedgcock) Moreau, Rev. Mycol. (Paris) Suppl.Col.17: 22, 1952.

≡ *Ceratostomella moniliformis* Hedgcock, Mo. Bot. Gard. Ann. Rept. 17: 78, 1906.
≡ *Ophiostoma moniliforme* (Hedgcock) Davidson, J. Agric. Res. 50: 800, 1935.
≡ *Endoconidiophora bunae* Kitajima, Bull. Imp. For. Exp. Sta., Meguro, Tokyo 35: 126.
= *Ceratocystis bunae* (Kitajima) Moreau, Rev. Mycol. (Paris) Suppl. Col. 17: 22, 1952.
= *Ceratocystis wilsonii* Bakshi, Mycol. Pap. 35: 4, 1951.
= *Ceratocystis filiformis* Roldan, Phillip. J. Sci. 91: 418, 1962.

Ceratocystis moniliformis was discovered in 1903 on gumwood (*Liquidambar styraciflua)* in Texas (Von Schrenk 1903). Hedgcock (1906) provided the first description of this fungus as *Ceratostomella moniliformis*. Although there have been a number of morphological descriptions of *C. moniliformis*, these differ quite markedly. *Ceratocystis moniliformis* has a few distinguishing characteristics that separate it from other species in this genus. One of these characteristics is the ornamentation on the ascomatal bases, which are covered in conical spines (Hedgcock 1906, Luc 1952, Hunt 1956, Upadhyay 1981). Another characteristic is the disciform shape of the base of the ascomatal neck (Bakshi 1951). This fungus is renowned for its rapid growth. Within three to five days, ascomata are formed from a single spore isolate and they mature within another one to two days (Hedgcock 1906, Davidson 1935). A characteristic banana-oil odour is produced when the cultures are young (Davidson 1935). Both cylindrical and barrel-shaped conidia are formed (Davidson 1935, Kitajima 1936, Bakshi 1951, Luc 1952).

Ceratocystis moniliformis infects wounds on many woody plants. Due to the fact that it is not a pathogen and only causes sapstain, the occurrence of the fungus has not been well documented. *Ceratocystis moniliformis* has been isolated in areas such as South Africa on *Macaranga capensis* Baill. (Van Wyk *et al.* 1991) and from much cooler climates such as Poland where it occurs on *Quercus robur* L. (Kowalski & Butin 1989).

10.5 *Ceratocystis moniliformopsis* Yuan & Mohammed *Austr. System. Bot.* 15: 125, 2002.

Ceratocystis moniliformopsis was described in 2002 from infected *Eucalyptus obliqua* L'Herit. logs in Australia (Yuan & Mohammed 2002). It is morphologically similar to *C. moniliformis*, as indicated by its name. The ascomata as well as ascospores are similar to those of *C. moniliformis* but it has a unique conidiogenous cell morphology, not found in other *Ceratocystis* spp. The pathogenicity of this fungus is still unknown.

10.6 *Ceratocystis acericola* Griffin *Can. J. Bot.* 46: 694, 1968.

Ceratocystis acericola was described in 1968 as a new species of *Ceratocystis* isolated from *Acer saccharum* Marsh in Ontario, Canada. The ascomatal bases are black, globose and ornamented with thick-walled hyphae (Griffin 1968). The ascomatal necks have a characteristic swelling or "annulus" below the apex with no ostiolar hyphae present

(Griffin 1968). The ascospores were first described as being orange-section shaped with a sheath (Griffin 1968) but later it was described as having hat-shaped ascospores (Grylls & Seifert 1993). No description of the anamorph was given.

11. CONCLUSIONS

The genus *Ceratocystis* includes fungi with variable ecological and pathogenic properties ranging from non-pathogenic staining fungi to highly aggressive pathogens (Wingfield *et al.* 1993). During the course of the last ten years, reports of *Ceratocystis* spp. associated with commercial plantation forestry in the Southern Hemisphere have increased dramatically. At least two of these species, *C. fimbriata* and *C. albofundus* are pathogenic to *Eucalyptus* spp. (Roux *et al.* 2000, Barnes *et al.* 2003b), and *A. mearnsii* (Morris *et al.* 1993, Roux, Dunlop & Wingfield 1999, Roux *et al.* 2001), respectively. The role of other *Ceratocystis* spp., as the causal agents of disease of exotic plantation forest trees, remains to be determined.

There are still many unanswered questions pertaining to the origin and dispersal of *Ceratocystis* species between countries and continents. Obtaining knowledge on these aspects could significantly contribute to the way the forestry industry could restrict further distribution of these pathogens. This also will impact on how disease management strategies could be formulated in future. The aim of this thesis is to study some *Ceratocystis* spp. in more detail that are important to the forestry industry. This study concentrates mainly on the morphology, phylogeny, pathogenicity and population structure of these fungi. Isolates included for this study are all characterised by having hat-shaped ascospores as was discussed in this review.

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13. APPENDIX

Table 1. Distribution and host range of *Ceratocystis moniliformis* causal agent of vascular stain.

Geographical distribution	Host	Reference
South Africa	*Macaranga capensis*	Van Wyk *et al.* 1991
France, Madagascar	*Theobroma* spp.	Luc 1952, Paulin-Mahady *et al.* 2002
Poland	*Quercus robur*	Kowalski & Butin 1989
USA	*Liquidamber styraciflua*	Von Schrenk 1903
"	*Pinus ponderosa*	Hedgcock 1906
"	*Pinus palustris, P. echinata, P. taeda, Liquidamber styraciflua, Liriodendron tulipifera, Nyssa aquatica, Fagus grandifolia, Magnolia* sp., *Quercus* sp.	Davidson 1935
Tokyo	*Fagus crenata, Quercus glandulifera, Magnolia hyplleuca, Kalopanax ricinifolius, Ptercarya rhoifolia, Cercidiphyllum japanicum*	Kitajima 1936
Scottland	*Quercus* sp.	Bakshi 1951
Cameroon	*Pycnanthus kombo*	Luc 1952
Madagascar	*Theobromae* spp.	Luc 1952
Philippines	*Calamus maximus, Endospermum peltatum, Parkia javanica*	Roldan 1962
China	*Hevea* sp.	Witthuhn *et al.* 1999
South Africa	*Erythrina* sp.	Witthuhn *et al.* 1999

Table 2. Distribution and host range of *Ceratocystis fimbriata*.

Geographical distribution	Host	Reference
AFRICA		
Republic of Congo, Uganda, Kenya	*Eucalyptus* spp., *Ipomoea batas*	Khiurani, Carey & Narla 2000, Roux *et al.* 2000
ASIA		
Taiwan, South East Asia, China	*Crotolaria juncea, Ipomoea batas, Punica granatum, Mangifera indica*	Chun Yee *et al.* 1997, Clark & Moyer 1988, Huang *et al.* 2003
AMERICAS		
Brazil, Colombia, USA, Central and South America, Venezuela, Canada, Uraguay	*Crotolaria juncea, Ipomoea batas, Populus* spp., *Hevea brazilienis, Prunus* spp., *Citrus* spp. *Platanus* spp., *Coffea arabica, Theobroma cacao, Acacia* spp., *Gwemelina arborea, Eucalyptus* spp., *Mangifera indica*	Halsted 1890, Beeley 1929, Sharples 1936, Costa & Krug 1935, Pontis 1951, Walter, Rex & Schreiber 1952, Iton 1959, Zalasky 1965, Manion & French 1967, DeVay *et al.* 1968, Muchovej, Albuquerque & Riberio 1978, Vigouroux 1979, Panconesi 1981, Riberio *et al.* 1985, Toviotdale & Harper 1991, Mourichon 1994.
AUSTRALIA		
Australia	*Syngonium* spp.	Walker *et al.* 1988
EUROPE		
Europe, France, Italy, Spain, Switzerland	*Populus* spp., *Platanus* spp.	Walter *et al.* 1952, Panconesi 1981

Table 3. Important species of *Ceratocystis* pathogenic to trees, crops, and ornamental shrubs.

Ceratocystis species	Host	Disease	Reference
C. fimbriata	Sweet potato	Rot	Halsted 1890
"	Coffee & Rubber trees	Wilt	Pontis 1951,
"	Eucalypt	Canker	Roux *et al.* 2000
C. paradoxa	Palms & Sugarcane	Root- and stem rot	Moreau 1952, Hausner *et al.* 1993a
C. adiposa	Sugarcane	Root rot	Moreau 1952, Hausner *et al.* 1993a
C. fagacearum	Oak trees	Vascular wilt	Hunt 1956, Olchowecki & Reid 1974, Hausner *et al.* 1993a
C. polonica	Spruce trees	Vascular wilt	Yamaoka *et al.* 1997
C. virescens	Maple trees	Sap streak	Olchowecki & Reid 1974
C. albofundus	Wattle trees	Vascular wilt	Morris *et al.* 1993. Roux *et al.* 1999

Table 4. Morphology of the teleomorph of *Ceratocystis* spp. with hat-shaped ascospores.

PERITHECIAL	*C. moniliformis* (Hedgcock 1906)	*C. fimbriata* (Upadhyay 1981)	*C. pirilliformis* (Barnes *et al.* 2003)	*C. moniliformopsis* (Yuan & Mohammed 2002)	*C. albofundus* (Wingfield *et al.* 1996)	*C. acericola* (Griffin 1968)
BASE:						
Colour	Brown → black	Dark brown →black	Black	Dark brown →black	Yellowish brown	Black
Diameter	90 – 180 µm	950 – 1200 µm	115 – 187 µm	200 – 320 µm	416 – 848 µm	75 – 100 µm
Ornamentation	Spares conical spines, 12 – 16 µm	Few hyphal hairs	Hyphae	Pigmented setae, hyphae (base)	Unornamented	Hyphae
Form	Globular	Globose	Pear-shaped	Oviod	Globose	Globose
NECK:						
Colour	Black	Black (base) → lighter (tip)	Black	Black	Black (base) → light (tip)	-
Base	Disciform	-	-	Disciform	Collar	-
Length	500 – 900 µm	950 µm	327 – 683 µm	480 – 780 µm	208 – 840 µm	100 – 400 µm
Width: base	20 – 40 µm	18 – 35 µm	19 – 33 µm	40 – 50 µm	20 – 32 µm	20 – 35 µm
Width: tip	10 – 15 µm	10 – 18 µm	12 – 21 µm	18 – 22 µm	16 – 24 µm	8 – 12 µm
Ostiolar hyphae	Brown, strait, 12 – 18 µm	Hyaline, straight or flexuous, tapered, blunt or subulate, 18 – 75 µm	Hyaline, strait, convergent	Hyaline, aseptate, unbranched, convergent 25 – 45 x 1.5 – 2 µm	Hyaline, divergent 40 – 60 µm	None
ASCUS:	Evanescent	Not seen	Evanescent	Not seen	Evanescent	Not seen
ASCOSPORES:						
Colour	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline
Shape: face view	Oval	Elliptical	Elliptical	Oblong	Elliptical	Cylindrical
Shape: side view	Flat on 1 side	Reni-form	Hat-shaped	Reniform / Hat-shaped	Hat-shaped	Orange section
Shape: end view	-	Oval	-	-	-	Globose to elliptical
Length	4 – 5 µm	3.5 – 8 µm	8 – 11.5 µm	4 – 5 µm	4 – 6 µm	2.5 – 4.0 µm
Width	3 – 4 µm	2 – 2.5 µm	4 – 6 µm	2 – 2.5 µm	3.5 – 5 µm	1.5 – 2.5 µm
Texture	Slimy, grey mass	Tawney, mucilaginous cap	Hyaline, gelatinous sheath	Hyaline, gelatinous sheath	Slimy droplet, double brim	Hyaline, gelatinous sheath

Table 5. Morphology of the anamorph of *Ceratocystis* spp. with hat-shaped ascospores.

	C. moniliformis (Hedgcock 1906)	*C. fimbriata* (Upadhyay 1981)	*C. pirilliformis* (Barnes *et al.* 2003)	*C. moniliformopsis* (Yuan & Mohammed 2000)	*C. albofundus* (Wingfield *et al.* 1996)
CONIDIOPHORES:					
Length	-	35 – 130 µm	62 – 147 µm	5 – 32.5 µm	24 – 104 µm
Width	-	2.5 – 6 µm (base)	-	4 – 5.3 µm	3 – 5 µm
CONIDIA:					
Shape	Cylindrical	(1) Cylindrical (2) Barrel	(1) Cylindrical (2) Barrel	(1) Cylindrical (2) Barrel	(1) Cylindrical (2) Barrel
Length	6 – 8 µm	(1) 9 – 21 µm (2) 9 – 14 µm	(1) 12 – 25 µm (2) 4 – 6 µm	(1) 13 – 21 µm (2) 12 – 17.5 µm	(1) 8 – 24 µm (2) 6 – 10 µm
Width	1.8 – 2.2 µm	(1) 2 – 6.5 µm (2) 5 – 15 µm	(1) 2 – 4 µm (2) 3 – 4 µm	(1) 2 – 3 µm (2) 5 – 7.5 µm	(1) 3 – 4 µm (2) 4 – 8 µm
CHLAMYDOSPORES:	No	Yes	Yes	No	No
Length	-	9 – 15 µm	8 – 12 µm	-	-
Width	-	7.5 -11 µm	5 – 8 µm	-	-
CULTURE:					
Growth rate	Perithecia in 2 – 3 days	45 – 55 mm in 12 days	22 mm in 12 days	6 – 7.5 mm per day	27 mm in 8 days
Colour	Hyaline → grey → black	Hyaline → grey/brown → brown/black	Hyaline → grey → green	Hyaline → green/brown	White → creamy
Mycelium	Coarsely granular, 2 – 8 µm dia.	Smooth, 1.5 – 5 µm dia.	-	-	Hyaline, smooth, 2 – 5 µm dia.