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Review

Life styles of *Colletotrichum* species and implications for plant biosecurity

Dilani D. DE SILVA[a], Pedro W. CROUS[c], Peter K. ADES[d], Kevin D. HYDE[b], Paul W. J. TAYLOR[a],*

[a]Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC, Australia
[b]Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand
[c]Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands
[d]Faculty of Science, The University of Melbourne, Parkville, VIC, Australia

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ABSTRACT

Colletotrichum is a genus of major plant pathogens causing anthracnose diseases in many plant crops worldwide. The genus comprises a highly diverse group of pathogens that infect a wide range of plant hosts. The life styles of *Colletotrichum* species can be broadly categorised as necrotrophic, hemibiotrophic, latent or quiescent and endophytic; of which hemibiotrophic is the most common. The differences in life style depend on the *Colletotrichum* species, the host species, the physiological maturity of the host and environmental conditions. Thus, the genus *Colletotrichum* provides a unique opportunity for analysing different life style patterns and features underlying a diverse range of plant–pathogen interactions. This review describes the various modes of life styles of *Colletotrichum* species, the underlying mechanisms of infection and colonisation, and implications the life styles have for plant biosecurity. Knowledge of life styles of *Colletotrichum* species will enable the development of improved diagnostics and application of integrated disease control methods to mitigate the risk of incursion of exotic *Colletotrichum* species.

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1. Introduction

Plant-infecting fungi comprise a diverse group of organisms with different life styles and methods by which they infect and colonise their hosts (Agrios, 2004). Plant-fungus interactions have traditionally been categorised as pathogenic, parasitic or mutualistic in a broad sense. However, it is hard to identify clear examples of host-fungus relationships in nature that entirely fit these definitions (Newton et al., 2010). Most plant pathogenic fungi show a variety of interactions with their host plants, changing their relationship at different stages of their life cycle depending on the physiological maturity and resistance of the host, the environment and associated virulence genes of the pathogen (Stergiopoulos and de Wit, 2009; Newton et al., 2010; Delaye et al., 2013; Stergiopoulos and Gordon, 2014).

The genus *Colletotrichum* includes many important plant pathogens that cause major production losses in many plant

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crops worldwide (Agrios, 2004; Hyde *et al.*, 2009, 2014; Cannon *et al.*, 2012; Fig. 1). Mostly they are important as post-harvest pathogens of fruits and vegetables, but are also reported to cause diseases of leaves, stems, tubers and seedlings in the field (Damm *et al.*, 2012a, 2012b; Udayanga *et al.*, 2013). In general, *Colletotrichum* species show different life styles that vary between groups of species, with most species being able to sequentially switch between life styles (O'Connell *et al.*, 2012). The life style patterns found in *Colletotrichum* species can be broadly categorised as necrotrophic, hemibiotrophic, latent or quiescent and endophytic, of which hemibiotrophy is the most common (Table 1) (Perfect *et al.*, 1999; Peres *et al.*,

2005; Münch *et al.*, 2008; Auyong *et al.*, 2012; Barimani *et al.*, 2013).

Many *Colletotrichum* species belong to different species complexes containing mostly cryptic species that are closely related to each other and have similar infection and colonisation behaviour (Sanders and Korsten, 2003; Damm *et al.*, 2012a, 2012b; Jayawardena *et al.*, 2016). However, it should be noted that many of the *Colletotrichum* species referred to in older publications should be treated as *sensu lato* (in a broad sense) as many are known now to be complexes of numerous, cryptic species. As many *Colletotrichum* species can survive on a broad host range they can develop unique relationships with
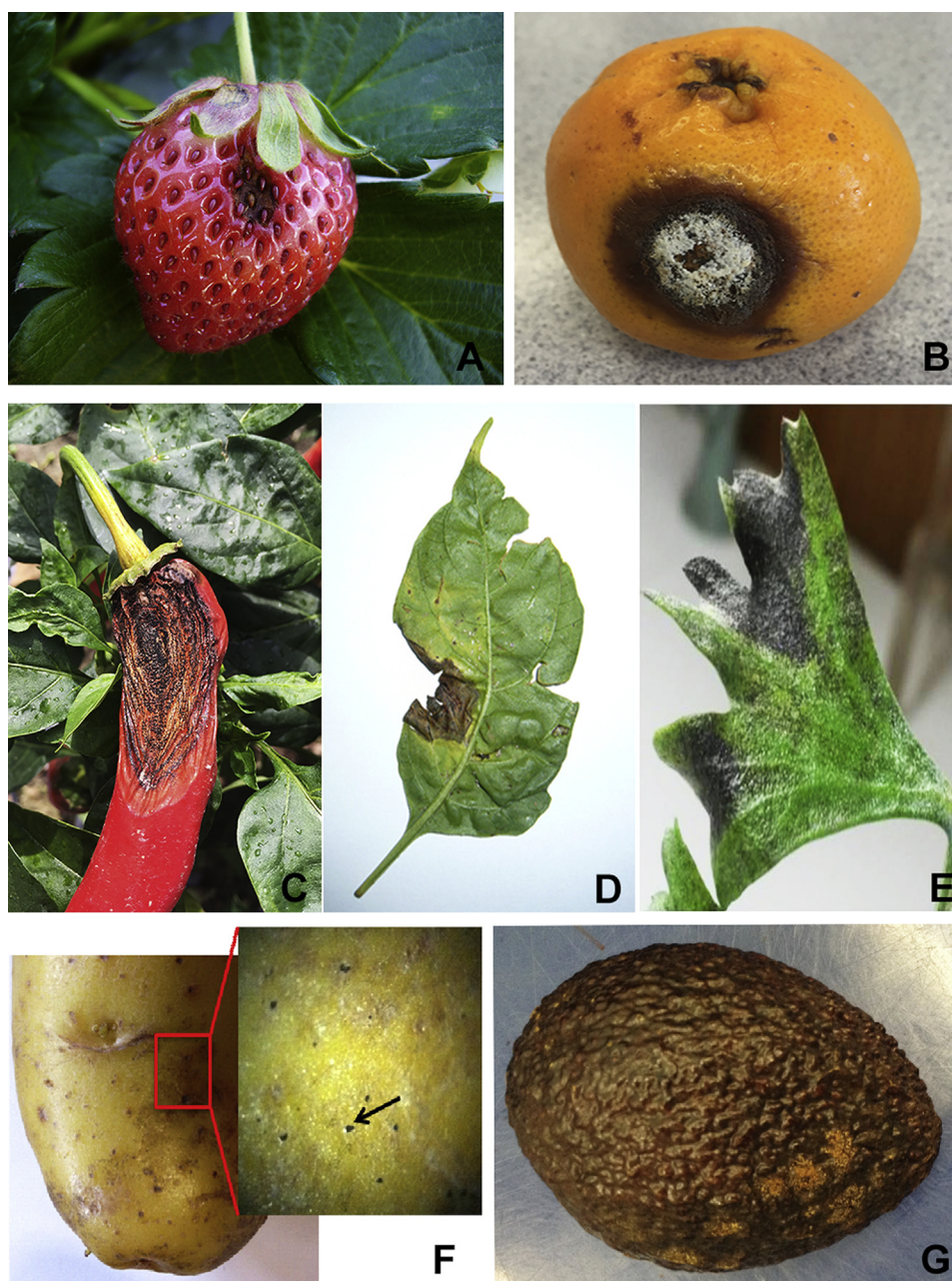
Fig. 1 − Anthracnose symptoms caused by *Colletotrichum* spp. A) *C. acutatum* on strawberry fruit, B), *C. gloeosporioides* on mandarin citrus C) and D) *C. truncatum* on chili fruit and leaves, E) *C. karstii* on pyrethrum leaves, F) *C. coccodes* on potato tuber showing micro sclerotia (arrow) and G) *C. gloeosporioides* on avocado.

Table 1 — Life styles of *Colletotrichum* species on different host plants.

Life style	*Colletotrichum* species/ complex	Host plant	Reference
Intracellular hemibiotrophy	Acutatum complex	Capsicum	Kim *et al*., 2004
Hemibiotrophic life style with a subcuticular, intramural necrotrophy with no detectable biotrophic stage	Acutatum complex	Strawberry	Curry *et al*., 2002 Arroyo *et al*., 2005
Hemibiotrophic life style with predominant biotrophic life style	Acutatum complex Acutatum complex	Citrus leaves Blueberry fruits	Zulfiqar *et al*., 1996 Wharton and Diéguez-Uribeondo 2004
Hemibiotrophic life style with intra cellular hemibiotrophy with predominant necrotrophic life cycle	*C. gloeosporioides*	Chili fruits	O'Connell *et al*., 2004
Hemibiotrophic life style with a subcuticular, intramural necrotrophy	*C. truncatum*	Chili leaves, fruit	Ranathunge *et al*., 2012
Localized hemibiotrophy life style with distinct multilobed infection vesicle	Destructivum complex (*C. tanaceti*)	Pyrethrum leaves	Barimani *et al*., 2013
	C. destructivum	Tobacco leaves	Shen *et al*., 2001
Extended hemibiotrophy life style	Graminicola complex	Cereals and grass species	Crouch and Beirn 2009
Endophytic life style	Boninense complex	Several tree species	Lu *et al*., 2004
	Gloeosporioides complex and Graminocola complex	Tropical grass species	Manamgoda *et al*., 2013
	C. coccodes	Potato stems, roots	Chang et al., 2014
Endophytic (quiescent) infection followed by necrotrophy	*C. truncatum* *C. coccodes*	Chili leaves, fruit	Ranathunge *et al*., 2012, Chang *et al*., 2014

particular hosts, not always associated with disease, but these asymptomatic infections may serve as inoculum sources for infection of other plants (Freeman *et al*., 2001; Peres *et al*., 2005; Yang *et al*., 2009; Phoulivong *et al*., 2012; Udayanga *et al*., 2013). The complex life cycles of many *Colletotrichum* species, their potential to cross infect a wide range of host species and to switch their life style create major difficulties in managing the diseases they cause (Phoulivong *et al*., 2012; De Silva *et al*., 2016b).

The life style patterns of *Colletotrichum* species are highly regulated by specific gene families and biochemical interactions that occur through specific enzymes and secondary metabolites produced at the host-pathogen interface. Genus-wide comparative genome analyses have shown that *Colletotrichum* species have tailored profiles of some enzymes according to their specific life style (Gan *et al*., 2016). Genomic and transcriptomic research over the last decade has provided insights into the genetic basis of the variable life styles of plant fungal pathogens and the importance of "gene-based biosecurity" (Crous *et al*., 2016; McTaggart *et al*., 2016; Zeilinger *et al*., 2016).

Understanding the complex life style patterns of *Colletotrichum* spp. and the dynamic state of their interactions with their hosts has important implications for disease control and plant biosecurity risk management (Fry, 1982; Newton *et al*., 2010; Bourget *et al*., 2013). Changes in life style across successive stages in the life cycle of *Colletotrichum* species can make their detection and control very difficult (Fig. 2). Management strategies should not be restricted to consideration only of symptomatic hosts, but also the pathogen's life style state where infected plants may not be showing symptoms. The fact that plant pathogens such as *Colletotrichum* species can be dispersed by transport of asymptomatic hosts over long distances is of major importance when considering quarantine measures (Strange and Scott, 2005; Newton *et al*., 2010). Knowledge of the different life style patterns of specific *Colletotrichum* species needs to be embedded in processes for developing biosecurity policy and proper estimation of the risk involved in trade and allowing importation of certain plant materials.
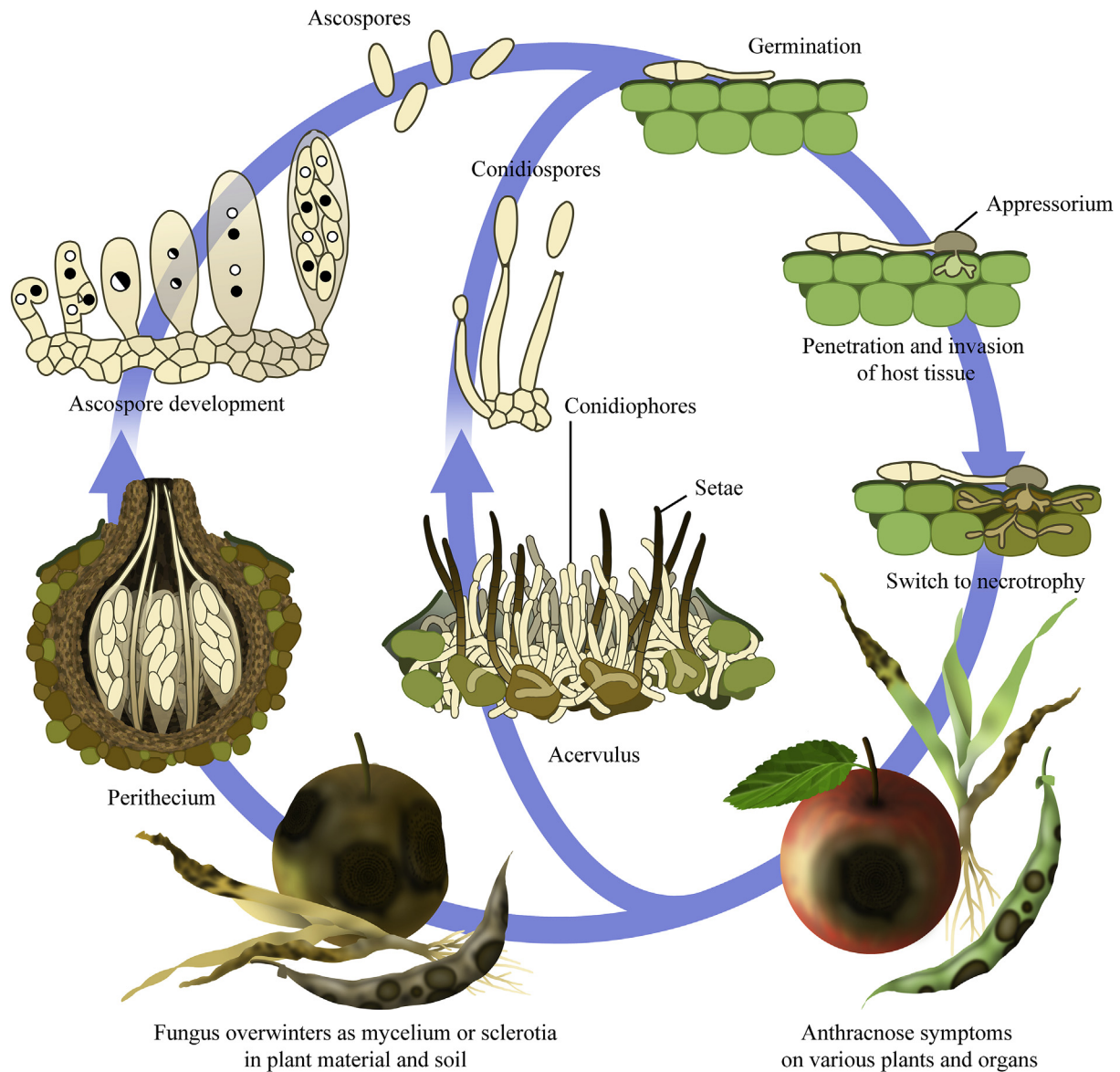
2. Life styles of *Colletotrichum* species

Necrotrophic life style

Necrotrophic pathogens are those that actively infect and colonise plant cells leading to cell death (van Kan, 2006). Necrotrophs typically secrete lytic enzymes to degrade plant components or toxins that kill the plant tissues. The pathogen subsequently survives on the contents of dead or dying cells to complete its life cycle (Stone, 2001; Kleemann *et al*., 2012; Gan *et al*., 2013). A necrotrophic life style contrasts with that of biotrophic pathogens which derive nutrients from living cells and therefore must maintain host viability (Kan, 2005). Nearly all *Colletotrichum* species develop a necrotrophic stage at some point in their life cycles except those few that exist entirely as endophytes (Prusky *et al*., 2013; Chang *et al*., 2014).

Biotrophic and hemibiotrophic life styles

A biotrophic life style in the strict sense is where the pathogen remains inside the living plant tissue and actively absorbs plant metabolites for its growth without killing the plant's cells (Mendgen and Hahn, 2002). Fungal biotrophs produce specialised fungal structures, haustoria, that are highly differentiated infection structures necessary for

Fig. 2 – General life cycle of *Colletotrichum* species.

pathogenesis and which facilitate the parasitic relationships with the living host plants to absorb carbon and nitrogen (Agrios, 2004; De Silva *et al.*, 2016b). True obligate, biotrophic fungi form haustoria and engage in long-term suppression of host defence responses (Voegele and Mendgen, 2011). Although the host plants remain alive, often without obvious disease symptoms, plant growth can be affected. To establish biotrophy in host plants and to successfully colonise plant cells pathogens need to secrete a variety of effector proteins that manipulate their host's physiological and biochemical environment mainly by suppressing plant defence responses (Dou and Zhou, 2012; O'Connell *et al.*, 2012; Gan *et al.*, 2013; Guyon *et al.*, 2014). *Colletotrichum* species generally do not show true biotrophy. Biotrophy is more common in groups such as the rust and powdery mildew fungi, in which the fungi must complete their life cycles within living host tissue (obligate biotrophy) (Voegele and Mendgen, 2011).

However, many *Colletotrichum* species can have a biotrophic stage early in their life style, followed by a switch to a necrotrophic life style, and thus are referred to as hemibiotrophs. For these species, primary infection vesicles are formed during initial infection of the epidermal cells without killing the cells. This is followed by a necrotrophic stage in which secondary infection hyphae invade and kill adjacent cells (Perfect *et al.*, 1999; Barimani *et al.*, 2013). The degree of hemibiotrophy varies among different *Colletotrichum* species according to their typical life style pattern and the timing of the switch from biotrophy to necrotrophy depends on host development stage and environmental conditions (Wharton and Diéguez-Uribeondo, 2004; Arroyo *et al.*, 2005; Peres *et al.*, 2005; Crouch and Beirn, 2009; Ranathunge *et al.*, 2012).

Quiescent life style

Quiescence (latency) defines an extended period of time in the fungal life cycle in which the pathogen exists dormant within the host before it switches to an active phase (Prusky *et al.*, 2013). This cannot be taken as a major life style phase however, it is a period of transition between the other phases of the life cycle. During quiescence, pathogen activity appears to be suspended and almost no growth occurs. This life style phase is common in pathogens causing postharvest disease of fresh fruit and vegetables especially ascomycetes (*Alternaria*, *Botrytis*, *Botryosphaeria*, *Diaporthe*, *Monilinia*, *Phyllosticta* and *Sclerotinia*) including *Colletotrichum* species. In these cases the pathogens remain dormant inside the plant tissue before disease symptoms develop, often after harvest, storage, transportation and sale of produce (Prusky, 1996; Adaskaveg and Förster, 2000; Wikee *et al.*, 2011; Gomes *et al.*, 2013; Prusky *et al.*, 2013; Kan *et al.*, 2014; Shaw *et al.*, 2016). However, the quiescent stage of the life style is difficult to define as each fungal species may show different transitions from quiescence to necrotrophy. Physiological and biochemical changes during the fruit ripening process may activate different defensive signal-transduction pathways related to both host and pathogen responses, which are important in maintaining or facilitating the transition from the quiescent to the necrotrophic life style (Prusky *et al.*, 2013).

Some *Colletotrichum* species which show a quiescent period in their life style before causing postharvest disease of fruits are *C. gloeosporioides* in avocado (Prusky, 1996) and *C. acutatum* in almond (Adaskaveg and Förster, 2000). A detailed study of *C. acutatum* and *C. gloeosporioides* infection on olives showed that the conidia of the pathogen germinated, formed appressoria and then remained dormant on non-targeted vegetative organs such as leaves and branches, which served as a source of overwintering inoculum (Talhinhas *et al.*, 2011). Once environmental conditions became favourable, infection developed and spores were dispersed via wind or rain to all plant organs. Trichomes and thick cuticles of leaves and branches may have reduced the infection on these non-targeted organs. Nevertheless, unripe fruit also became infected through penetration of the cuticle, but further colonisation of the plant cells did not occur until the fruit started to ripen. Colonisation then occurred, with multi-lobed hyphae produced inside host cells, typical of a hemibiotrophic life style (Talhinhas *et al.*, 2011). These findings suggested that *Colletotrichum* species became quiescent after infection then became active and started to colonise the cells when the environmental conditions and host physiology were conducive to completing the disease cycle.

Ranathunge *et al.* (2012) reported a quiescent stage in *C. truncatum* after initial infection of chili (*Capsicum annuum*) fruit with no apparent symptoms until six days after inoculation. Symptoms around the inoculation site were associated with intramural hyphae colonising the walls of collenchyma cells of the pericarp, prior to invading these cells and progressing to a necrotrophic phase. Moreover, a much longer quiescent stage occurred in infected young leaves of chili plants with no symptoms apparent until leaves matured and started to senesce naturally, at which time acervuli formed and provided primary inoculum for subsequent fruit infection.

Endophytic life style

Endophytic fungi are plant inhabiting fungi that live within the host plant cells as symbionts without causing apparent disease (Wilson, 1995; Rodriguez *et al.*, 2009; Vieira *et al.*, 2014; Hardoim *et al.*, 2015). They are taxonomically diverse in nature and common in almost every vascular plant (Arnold, 2007; Lima *et al.*, 2012). Most endophytic fungi have a strong mutualistic relationship with their host in which the fungi benefit from having a niche, with protection from desiccation and access to nutrients and photosynthetic products. In return, the host may benefit in various ways as fungal endophytes may improve host growth, enhance the plant's ability to tolerate abiotic stresses, as well as increase resistance to herbivores and pathogens (Gao *et al.*, 2010; Porras-Alfaro and Bayman, 2011; Kivlin *et al.*, 2013; Busby *et al.*, 2016).

Evidence suggests that the interaction between host plant and the endophytic fungus can sometimes switch from mutualistic to antagonistic or pathogenic depending on the plant's physiological condition, host genotype and the environmental conditions (Photita *et al.*, 2004; Promputtha *et al.*, 2007; Hardoim *et al.*, 2015). A recent study on evolution of fungal interactions found that most endophytic species were phylogenetically close to species with typical necrotrophic life styles, and in most fungal lineages species had switched between endotrophy and necrotrophy multiple times in the evolutionary and ecological timescale (Delaye *et al.*, 2013). More importantly, the ability of some isolates to grow asymptomatically inside the host in a endophytic manner could support the speculation that plant–pathogen interactions started from the breakdown of early mutualistic interactions to a pathogenic life style (Stukenbrock and McDonald, 2008; Toby Kiers *et al.*, 2010; Eaton *et al.*, 2011).

Many *Colletotrichum* species have been shown to exist as endophytes for part or most of their life cycles in many groups of plants (Rojas *et al.*, 2010; Rivera-Orduna *et al.*, 2011; Cannon *et al.*, 2012; Manamgoda *et al.*, 2013). The majority of *Colletotrichum* isolates that have been reported as endophytes fall within the gloeosporioides, graminicola and boninense species complexes (Bhagya *et al.*, 2011; Damm *et al.*, 2012b; Weir *et al.*, 2012; Vieira *et al.*, 2014). In particular, many endophytes isolated from grasses (*Poaceae*) belonged to the gloeosporioides and boninense complexes (Saikkonen *et al.*, 2000; Sánchez_Márquez *et al.*, 2010; Ghimire *et al.*, 2011; Liu *et al.*, 2015). Several new *Colletotrichum* species with endophytic life styles were identified on many temperate and tropical grass species (Pimentel *et al.*, 2006; Guo *et al.*, 2008; Mouhamadou *et al.*, 2011; Crouch and Beirn, 2009; Manamgoda *et al.*, 2013).

Thirty-nine endophytic *Colletotrichum* isolates of *C. gloeosporioides sensu lato*, *C. boninense* and *C. simmondsii* were isolated from Brazilian pepper trees (*Schinus terebinthifolius* Raddi. Anacardiaceae), with high intraspecific genetic diversity within the *C. gloeosporioides* group (Lima *et al.*, 2012). Rojas *et al.* (2010) found that most of the *Colletotrichum* species associated with Cocoa plants (*Theobroma cacao*) and other plants in Panama separated into two distinct clades: one which contained asymptomatic endophytes with a range of host species including native Panamanian trees and the other which comprised pathogenic species, generally restricted to

single host species. *Colletotrichum tropicale* and *C. ignotum* were found to be frequent asymptomatic associates of cacao and other neotropical plant species, while *C. theobromicola* was associated with foliar and fruit anthracnose lesions of cacao but not isolated from any other hosts. These results suggest that pathogens have a specialised relationship with the host plants thus are more restricted in host range while endophytic species occur in multiple host species. Moreover, Lu et al. (2004) identified endophytic *Colletotrichum* strains belonging mostly to gloeosporioides and boninense complexes in leaves of 12 tree species from a forest in Guyana. Manamgoda et al. (2013) isolated and identified four endophytic species belonging to the gloeosporioides complex − *C. endophytica*, *C. fructicola*, *C. siamense* and *C. tropicale* from the grasses *Cymbopogon citratus* and *Pennisetum purpureum* in Thailand. However, *C. fructicola* and *C. siamense* have also been reported as necrotrophic pathogens in other plant species such as *Capsicum annuum*, which highlights the lack of host specificity in endophytic species of *Colletotrichum* (Sharma and Shenoy, 2014).

A phylogenetic approach using maximum-likelihood analysis, combined with ancestral character mapping showed that most endophytic fungi including *Colletotrichum* species had a close relationship to species with a necrotrophic life style and life styles had been switched multiple times during pathogen evolution (Delaye et al., 2013). Several studies have reported shifts from endophytic to pathogenic life style in fungal species (Álvarez-Loayza et al., 2011; Eaton et al., 2011). An example of a switch between endophytic and necrotrophic life styles was shown in *C. coccodes* a pathogen with a wide host range especially in solanaceous plant species (Chesters and Hornby, 1965; Lees and Hilton, 2003). *Colletotrichum coccodes* has been isolated as an endophyte from healthy, symptomless plants of potato, cabbage, white mustard, lettuce and chrysanthemum (Chesters and Hornby, 1965; Chang et al., 2014). In potato, *C. coccodes* causes black dot of the tubers. Chang et al. (2014) showed that leaves of potato plants inoculated with *C. coccodes* isolates originating from infected tubers showed no symptoms after infection although appressoria and primary infection hyphae were present (Fig. 3A).

However, 12 weeks after inoculation the pathogen had colonised the stems, roots, stolon and tubers indicating an extensive endophytic life style. The pathogen moved rapidly through the vascular system to colonise below-ground parts but none of the plant tissue expressed necrotic symptoms. Leaf lesions only develop on detached leaves or leaves *in vivo* after being inoculated with a very high spore concentration (10^6 spores/mL). In Australia, the major economic loss caused by black dot disease is the deterioration of tuber quality and loss of market value due to skin blemishes on the tubers, with leaf or stem lesions being rare. Interestingly, the same potato isolates of *C. coccodes* were able to infect wounded chili fruit and caused necrotic lesions similar to those of *Colletotrichum* spp. recognised as pathogens of chili. Hence, *C. coccodes* appears to have different mechanisms for infection and colonisation of different hosts and can switch from an endophytic to necrotrophic life style depending on the host species.

3. Infection and colonisation

Pre-infection

Penetration and colonisation of host tissues by *Colletotrichum* spp. generally starts with the germination of conidia and the formation of specialised infection structures, appressoria, which facilitate entry through the host cuticle and epidermal cell walls with the aid of narrow penetration pegs (Fig. 3) (Perfect et al., 1999; Wharton and Schilder, 2008). However, in rare cases direct penetration through stomata or wounds without formation of appressoria has been reported (Latunde-Dada et al., 1996; Zulfiqar et al., 1996; Wharton and Diéguez-Uribeondo, 2004). Interestingly, a study of the infection process of *C. acutatum* in *Capsicum* fruit found that the production of highly branched, thick-walled, dendroid structures facilitated the penetration at the cuticle (Liao et al., 2012). In contrast, during infection of mulberry leaves by *C. gloeosporioides*, the cause of black spot disease, no appressoria were produced, nor was there direct penetration through the epidermis. Instead, specialised infection vesicles formed
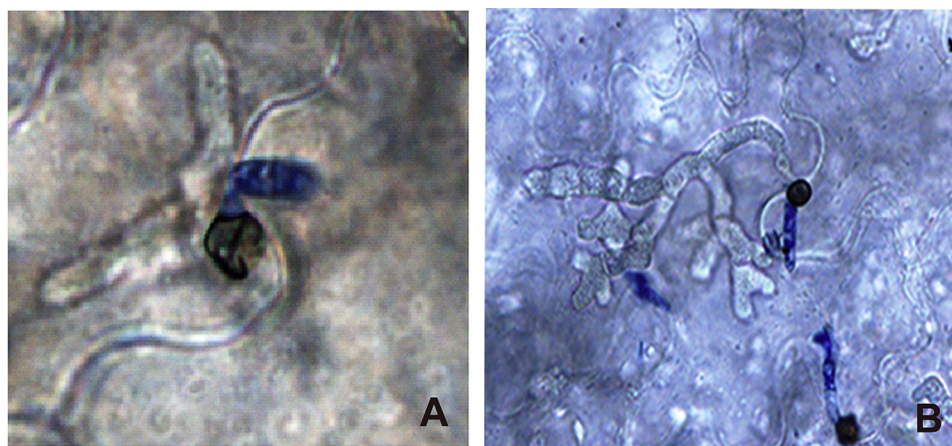
Fig. 3 − Leaf Infections caused by *Colletotrichum* spp A) *Colletotrichum coccodes* infection of a potato leaf showing an appressorium with direct penetration of an epidermal cell B) *C. lentis* infection of lentil leaf showing the germinated conidiospores with appressoria and a multi-septate, lobed infection vesicle.

over or within the stomata and the infection hyphae entered the leaves through the stomatal openings (Kumar *et al.*, 2001). The structure of the host plant surface (cuticle and epidermis thickness, leaf hairs, stomata, and trichomes) is also important in the initial infection process and may act as a barrier against fungal infection (Calo *et al.*, 2006; Serrano *et al.*, 2014).

Post Infection and colonisation

Post infection strategies adopted by many *Colletotrichum* species are either intracellular hemibiotrophy (Fig. 4A) or subcuticular, intramural necrotrophy (Fig. 4B) (Shen *et al.*, 2001; Wharton *et al.*, 2001; Wharton and Diéguez-Uribeondo, 2004; Münch *et al.*, 2008; O'Connell *et al.*, 2012). In intracellular hemibiotrophy the early stage of infection is the symptomless biotrophic phase in which the penetration peg invades the epidermal cells and the primary hyphae produce enlarged infection vesicles inside epidermal and mesophyll cells. At this stage the host cells stay alive and plants do not show any symptoms. This is followed by the necrotic phase in which thin secondary hyphae grow intracellularly and intercellularly while secreting cell wall degrading enzymes that kill the host cells (O'Connell *et al.*, 2012). In subcuticular, intramural necrotrophic infection the fungus grows under the cuticle within the periclinal and anticlinal walls of the epidermal cells without penetrating the protoplasts. Even though this is called subcuticular, intramural necrotrophic infection there may also be a very short biotrophic phase before intramural hyphae aggressively initiate the destruction of the colonised host tissues (Wharton and Diéguez-Uribeondo, 2004; Arroyo *et al.*, 2005; Peres *et al.*, 2005).

For species within the acutatum complex, infection strategies are based on both intracellular hemibiotrophy and subcuticular, intramural necrotrophy. However, for the intracellular hemibiotrophic strategy, transition from one state to another can vary depending on the host plant, host maturity stage and infecting *Colletotrichum* species, some of which can have unique infection behaviours. Species in the acutatum complex commonly start their life cycle with a short biotrophic phase before switching to a necrotrophic stage. In contrast species in the graminicola complex such as *C. sublineola* have an extended biotrophic phase before switching to the necrotrophic phase (Freeman *et al.*, 2001; Peres *et al.*, 2005; Kleemann *et al.*, 2012).

Many studies have described the infection processes of putatively named *C. acutatum* strains in several plant species (Zulfiqar *et al.*, 1996; Curry *et al.*, 2002; Wharton and Diéguez-Uribeondo, 2004). Peres *et al.* (2005) summarised the different infection modes including the predominant necrotrophic life style with a subcuticular, intramural infection stage but no detectable biotrophic stage on strawberry where the pathogen formed an intramural infection vesicle between the epidermal cell walls (Curry *et al.*, 2002; Arroyo *et al.*, 2005; Guidarelli *et al.*, 2011); a predominantly biotrophic life style on citrus leaves, with secondary formation of conidia (Zulfiqar *et al.*, 1996); and a hemibiotrophic life style comprising a mostly biotrophic phase on blueberry fruits (Wharton and Diéguez-Uribeondo, 2004). Dieguez-Uribeondo *et al.* (2005) reported hemibiotrophic and subcuticular, intra- and intercellular infection followed by a necrotrophic life style on almond leaves and fruits. On olive fruits, a combination of a biotrophic phase (unripe fruits) and necrotrophic phase (ripe fruits and on leaves) was reported, resulting in intra- and intercellular colonisation of the
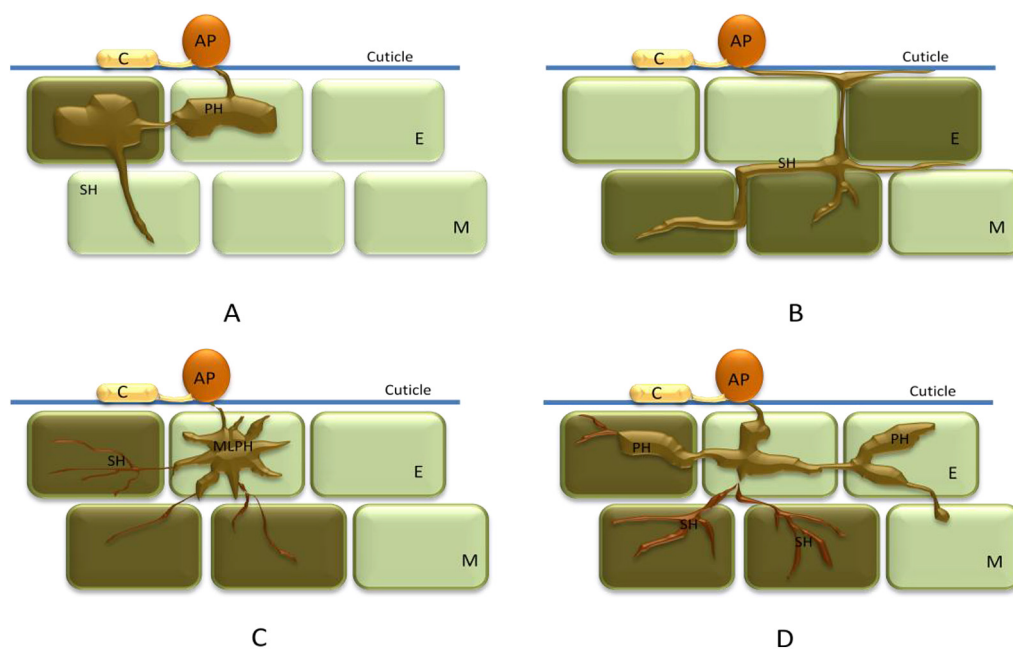
Fig. 4 — **Post infection strategies adopted by *Colletotrichum* species A: Intracellular hemibiotrophy B: subcuticular, intramural necrotrophy C: localized hemibiotrophic infection with multilobed primary hyphae D: Extended hemibiotrophy. C: conidia, AP: appressoria E: epidermal cells, M: mesophyll cells, PH: Primary hyphae, SE: Secondary hyphae, MLPH: multilobed primary hyphae.**

pericarp cells in the fruit (Gomes *et al.*, 2009, 2012). A study on infection strategies on different cultivars of blueberry fruit found that the pathogen modified its life style according to the susceptibility of the host tissue being colonised (Wharton and Schilder, 2008). However, the differences in infection strategies reported above may have been associated with several different *Colletotrichum* species within the acutatum, gloeosporioides or related complexes. The acutatum complex was recently revised using multigene phylogenetics resulting in the identification of many new species (Damm *et al.*, 2012a).

Infection by *C. gloeosporioides* was reported to be hemibiotrophic in which both intracellular hemibiotrophic and intramural necrotrophic infections were observed (O'Connell *et al.*, 2000; Kim *et al.*, 2004; Moraes *et al.*, 2013). The infection and colonisation strategy of *C. gloeosporioides* on guava fruit was identified as intracellular hemibiotrophic with formation of infection vesicle and hyphae at the initially infected epidermal cell, followed by a necrotrophic phase (Moraes *et al.*, 2013, 2015). Hemibiotrophic infection of chili fruit by *C. gloeosporioides* was shown to be followed by necrotrophic destruction of cells by lytic enzymes, and condensed cytoplasm through intercellular and intracellular colonisation (Kim *et al.*, 2004). Studies of taxonomy and pathogenicity of *Colletotrichum* species associated with olive anthracnose in different countries found that many species belonged to the gloeosporioides complex including *C. aenigma*, *C. gloeosporioides sensu stricto* (*s.s.*), *C. kahawae*, *C. queenslandicum*, *C. siamense* and *C. theobromicola*. Of these species, some were weakly pathogenic on olive fruits while *C. theobromicola* and some isolates of *C. gloeosporioides* were virulent on both green and ripening olives (Schena *et al.*, 2014). Fluorescence microscopy studies of the infection processes of *C. truncatum* infecting chili fruit revealed that this fungus was able to colonise through primary intramural hyphae branching through the cell walls (quiescent phase) in healthy fruit tissue without producing secondary biotrophic infection structures, before switching to the necrotrophic phase (Montri *et al.*, 2009; Mongkolporn *et al.*, 2010; Auyong *et al.*, 2012). Further studies to elucidate the life cycle of *C. truncatum* on chili plants demonstrated direct leaf cuticle penetration followed by intramural, quiescent and necrotrophic phases of colonisation (Ranathunge *et al.*, 2012).

The hemibiotrophic life style of pathogens in the destructivum complex are defined as having a localised hemibiotrophic infection stage in the initial stages of infection, in which an intracellular biotrophic phase is entirely confined within a single epidermal cell without extending into adjacent cells (Fig. 4C). These very large, extensively branched, multilobed primary hyphae that invaginate the plasma membrane are confined only to the initially penetrated cell. In a compatible host, this biotrophic phase with specialised primary hyphae lasts only 72 h after initial infection before shifting to a necrotrophic phase in which very narrow filamentous secondary hyphae grow from the tips of the primary hyphae to rapidly invade the surrounding cells causing necrotrophic anthracnose symptoms. The multi-lobed infection hyphae have been used to identify species in this complex. Latunde-Dada and Lucas (2007) found a close relationship in the infection process among isolates of several species in the

destructivum complex including *C. destructivum*, *C. linicola* and *C. lentis*. Other studies also confirmed the localised hemibiotrophic nature of the early infection in other species of the destructivum complex such as *C. tabacum*, *C. higginsianum* (O'Connell *et al.*, 2004) and *C. tanaceti* (Barimani *et al.*, 2013). Microscopic studies of the infection strategy of *C. pisicola*, *C. vignae* and *C. destructivum* (Bailey *et al.*, 1990; O'Connell *et al.*, 1993; Latunde-Dada *et al.*, 1996, 1997; Shen *et al.*, 2001) and *C. tanaceti* (Barimani *et al.*, 2013) identified multiseptate, multilobed infection vesicles in the epidermal cells (Fig. 3B).

Another group of *Colletotrichum* species that show an extended hemibiotrophic phase are isolates from the graminicola complex (Fig. 4D). Major species including *C. falcatum*, *C. graminicola*, *C. caudatum* and *C. sublineola* have an intracellular hemibiotrophic infection stage with a short period of biotrophy (Mims and Vaillancourt, 2002; Crouch and Beirn, 2009; Crouch, 2014). During this time the fungus grows between plant cell walls and plasma membranes without entering the cell. In *C. sublineola* there is formation of the biotrophic globular infection vesicles in epidermal cells with filamentous primary hyphae developing from these vesicles then colonising many other epidermal cells as intracellular mycelia. The same structures in *C. graminicola* (Mims and Vaillancourt, 2002) were explained as primary hyphae along with haustoria-like structures, that invaded many epidermal cells as well as mesophyll cells, becoming highly constricted at cell-to-cell penetration sites as they pass through host cell walls (Wharton *et al.*, 2001; Mims and Vaillancourt, 2002; Münch *et al.*, 2008). This is in contrast to species of the destructivum complex where the biotrophic multilobed infection vesicles and primary hyphae are confined to only the first infected cell (Wharton *et al.*, 2001; O'Connell *et al.*, 2004; Barimani *et al.*, 2013; Crouch and Beirn, 2009; O'Connell *et al.*, 2012). The necrotrophic infection phase occurs when secondary hyphae, which are distinctly thinner than primary hyphae, begin to spread throughout host tissue. In *C. sublineola*, necrotrophy began 66 h after penetration of sorghum (Wharton *et al.*, 2001) and for *C. graminicola* 48–72 h after penetration maize leaves (Mims and Vaillancourt, 2002). At this point, the *Colletotrichum* species begin to produce various phytotoxins and other chemicals that degrade host tissue which rapidly becomes necrotic (Münch *et al.*, 2008).

The life cycle of *Colletotrichum* species also includes reproductive stages (including both sexual/teleomorph and asexual/anamorph) which occur on the host plant or in plant debris (Fig. 2) (Sutton, 1992). However, the sexual stage of *Colletotrichum* species is rarely observed in many important complexes and is poorly understood while the asexual stage is most commonly associated with diseases symptoms (Ishikawa *et al.*, 2010; Cannon *et al.*, 2012; Damm *et al.*, 2012a, 2012b; Barcelos *et al.*, 2014). However, sexual fruiting structures (perithecia) readily occur in cultures of species in the gloeosporioides, boninense, destructivum and graminicola complexes (Manandhar *et al.*, 1986; Vaillancourt *et al.*, 2000; Rodriguez *et al.*, 2005; Ishikawa *et al.*, 2010). In the gloeosporioides complex species such as *C. alienum*, *C. fructicola*, *C. queenslandicum*, *C. salsolae*, *C. clidemiae*, *C. kahawae* subsp. *cigarro*, and *C. ti* have been reported to produce perithecia in culture. Several species in the boninense complex formed the sexual stage in culture and on *Anthriscus* stems used to

induce perithecia production. In the destructivum complex there are two heterothallic species, *C. lentis* and *C. tanaceti* (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4255528/ Barimani et al., 2013) reported as forming sexual morphs by artificially crossing isolates. Ascospore size and shape can be useful species-level diagnostic features. Reports of species not reproducing should be treated with caution as cultured isolates may have lost the ability to form perithecia, perhaps associated with storage conditions, differences in the growth media and frequent subculturing. If the sexual stage is present, the pathogen sexually reproduces to form ascospores inside of asci, which results in formation of dark, long-necked perithecia. Both homothallic and heterothallic strains have been observed. Sexual reproduction enhances the genetic variation among species and assists in the aerial dispersal of ascospores (Vaillancourt et al., 2000; Rodriguez et al., 2005; Pinto et al., 2012). Perithecia can act also as survival structures which help pathogens to overwinter or survive periods without a susceptible host.

Environmental conditions favourable for the formation of perithecia induce the liberation of ascospores which infect neighbouring plant tissues. Ascospores germinate and infect plant cells, and then the infected plant tissue develops acervuli which produce masses of conidia on conidiophores. These conidia are disseminated by rain splash or wind onto healthy leaves, young fruit or blossoms. Upon infection the pathogen continues to produce conidia throughout the season resulting in a polycyclic disease cycle. Senescence of the host tissue may induce development of the sexual stage to restart the life cycle.

Progress has been made in recent years in delineating the taxonomy of different *Colletotrichum* species that cause anthracnose. Molecular phylogeny and genomics data have been able to distinguish *Colletotrichum* species complexes and identify genetically distinct subgroups. *Colletotrichum* species exhibit dynamic interactions with their host and have different life styles depending on their trophic level and the gradient of pathogenicity. However, the complexity in host pathogen interactions and ability to switch between different life style stages is undetermined for some *Colletotrichum* species—host interactions with only fragments of the process understood for the majority of these systems. The infection and colonisation process of newly established *Colletotrichum* species are largely yet to be investigated.

4. Significance of *Colletotrichum* life styles for plant biosecurity

The emergence of new infectious diseases and spread of plant pathogens over different geographical locations has become a major concern for plant biosecurity (Strange and Scott, 2005; Gullino, 2008; Waage and Mumford, 2008; Fisher et al., 2012). Plant diseases pose a serious and continuing threat to crop security, food safety, biodiversity and biosecurity for agriculture producing countries. Understanding the complex life style patterns of fungi and the dynamic state of pathogenic interactions with their hosts has important implications for disease control and risk management of exotic species.

Risk assessment for new exotic pathogens in a country or quarantine zone will be determined by the presence of host plants, movement of inoculum to new areas and suitability of the environment. In the absence of suitable environmental conditions it is unlikely that the pathogen will successfully establish. In addition, fungal characteristics, such as different spore stages, wide host range, tolerance to environmental conditions, multiple reproductive modes, and changing life style patterns will influence the emergence of disease. An exotic pathogen introduced to a new environment can adapt in different ways, (1) the same pathogen may be more or less damaging when it is introduced in a new environment; (2) the pathogen may rapidly adapt to that new environment and become more damaging; (3) the pathogen may hybridise with other species which may result in what is effectively a new, more damaging species. Many plant pathogens have the ability to undergo genetic recombination causing the emergence of pathogenic lineages and/or the formation of novel genetic diversity, which may lead to the genesis of new pathogens with increased pathogenicity (Brasier, 2001; Wingfield et al., 2001; Giraud et al., 2010; Fisher et al., 2012).

Colletotrichum and other well-established plant pathogenic genera such as *Fusarium*, *Phytophthora* and *Botrytis* have broad host ranges and an ability to switch between different life style stages, and thus pose an extra risk. The fact that some *Colletotrichum* spp. can be dispersed in asymptomatic hosts is of major importance when considering biosecurity measures. The risk of introduction of *Colletotrichum* species that have extended hemibiotrophic and quiescent phases of their life cycle can be underestimated or overlooked by quarantine inspections of commercially traded plant parts such as fruits, vegetables and bulbs. In addition, parts of a pathogen's life cycle can remain in plant tissues such as leaves, dead plant material or seeds in which disease symptoms are seldom obvious. These are potential sources of inoculum that pose a high quarantine risk in transportation of plant material to a different region or country.

Colletotrichum species complexes comprise a large number of species, with many new species being described and many more likely to exist (Rojas et al., 2010; Cannon et al., 2012; Phoulivong et al., 2012; De Silva et al., 2016a). Each species complex has individual species that may infect many host plants and in some cases the host plant may have yet to produce any apparent disease symptoms. Studies showing isolation of the same *Colletotrichum* species from different hosts, often as the first report in some host species demonstrate the potential for cross infection (Alahakoon et al., 1994; Bernstein et al., 1995; Freeman et al., 1998; Phoulivong et al., 2012; De Silva et al., 2016a). Many highly aggressive *Colletotrichum* species, particularly isolates from the gloeosporioides and acutatum species complexes have endophytic and quiescent stages and thus can remain undetected and uncontrolled in asymptomatic hosts. These can then be persistent sources of inoculum to infect and cause disease in different host plants or crops (James et al., 2014).

There are several examples of *Colletotrichum* species listed as quarantine pathogens by national biosecurity regulation authorities. *Colletotrichum lentis* that infects lentils in Canada has yet to be detected in Australia, hence it is a restricted pathogen in Australia; the slow-growing type of *C. acutatum*

that infects citrus in the USA has not been detected in Australia thus is on the biosecurity watch list. *Colletotrichum* species causing black spot in strawberry are widespread all over the world and are particularly a serious problem in commercial fruit production systems (Freeman and Katan, 1997). *Colletotrichum acutatum* was listed as a regulated plant quarantine pest by the European and Mediterranean Plant Protection Organisation (EPPO) and the EU Council until 2009 and currently, there are strict regulations and monitoring systems to prevent the incursion of this pathogen to European countries (Calleja *et al.*, 2013). Certain species within the acutatum complex that cause anthracnose fruit rot in California and the Northern hemisphere are still absent from Chile, Estonia and Scotland; and have restricted distributions in several other countries (Sreenivasaprasad and Talhinhas, 2005, Damm *et al.*, 2012b, CABI, 2016). *Colletotrichum kahawae* ssp. *kahawae*, the pathogen causing coffee berry disease, is of quarantine concern in Australia and China, as presently it has only been reported from the African continent (Cooke *et al.*, 2009, Q-Bank, 2016). *Colletotrichum theobromicola* (syn *C. fragariae*), *C. horii* and *C. truncatum* are declared as prohibited pathogens in Western Australia (DAFWA, 2016).

Accurate and fast identification of *Colletotrichum* species is critical for the establishment of appropriate quarantine regulations and practices (Crous *et al.*, 2016; Jayawardena *et al.*, 2016). With the advent of molecular-based taxonomy and phylogenetic studies many new *Colletotrichum* species have been identified and this has placed pressure on biosecurity staff to identify risks posed by these new species. There are many pathogenic *Colletotrichum* species, particularly in the acutatum and gloeosporioides complexes, which are known to be pathogenic on specific hosts and those that have broad host range. Therefore, there is urgent need to further study the newly established cryptic species to determine their host ranges and severity of disease and to decide if any of these are a significant risk for quarantine. Fast and accurate molecular protocols will also need to be developed that can easily distinguish the exotic *Colletotrichum* species from their close relatives.

Molecular techniques including taxonomic and phylogenetic analysis are necessary in the initial identification of species and delineating species boundaries. However, identifying a pathogenic species solely based on molecular data is not sufficient and there needs to be a polyphasic approach based on morphological characters, information about the pathogen's life cycle and pathogenicity to support identification of new species (Cai *et al.*, 2009). A recent case study of *C. siamense sensu lato* provided a good example of how species boundaries in plant pathogens should be delineated, and demonstrated the importance of employing such a polyphasic approach to identification (Silva *et al.*, 2012; Liu *et al.*, 2016). Although there are many studies focused on species identification and phylogenetic relationships, thorough knowledge on the molecular and genetic basis underlying different lifestyles of *Colletotrichum* species is still very sparse and further research is needed.

Emergence and development of plant diseases can be controlled through integrated disease management practices such as planting resistant host varieties and appropriate use of fungicides and seed treatments. However, for these practices to be effective there needs to be accurate identification of the pathogen, knowledge of the pathogen's life cycle, virulence, disease cycle and implementation of broad biosecurity/quarantine practices over national and international borders. In the event of a particular *Colletotrichum* species incursion, details of the pathogen's life cycle; such as alternative hosts, host-pathogen interaction, survival stage and environmental conditions required for epidemic development should be considered (Miles *et al.*, 2013; Verma *et al.*, 2007; Moraes *et al.*, 2015). Unravelling the life style and host interactions of *Colletotrichum* species will eventually lead to better understanding of the roles that the species play as pathogens, and hence will lead to improved disease management and biosecurity. By studying diverse life style patterns of *Colletotrichum* species, key processes in the host-pathogen interplay and information gained from molecular studies can be exploited for more sustainable disease control measures.

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