Infection of *Phaseolus vulgaris* with the plant pathogen *Colletotrichum* species

---

A thesis

submitted in partial fulfillment

of the requirements for the

Degree of Master

at

Lincoln University

by

Dorah Bloch Ayaa

Lincoln University

2021

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Agricultural Science

Infection of green bean, *Phaseolus vulgaris*, with the plant pathogen, *Colletotrichum* species

by

Dorah Bloch Ayaa

Colletotrichum gloeosporioides and *C. acutatum* are the main causal agents of mango anthracnose also infect multiple other hosts in tropical and subtropical regions of the world. Common bean, *Phaseolus vulgaris,* being a cover crop, is commonly grown with mango plants in Uganda. These co-planted annual crop species can play a role as potential inoculum reservoirs in mango orchard cropping systems. The purpose of this study was to identify *Colletotrichum* species isolates obtained from naturally infected apple and avocado fruits using traditional and molecular means, to determine the pathogenicity of *Colletotrichum* species on common bean, *Phaseolus vulgaris,* an annual crop grown concurrently in mango cropping systems, and to assess the survival of *Colletotrichum* species on *P. vulgaris* crop debris under glasshouse conditions at Lincoln University. Five *Colletotrichum* species isolates were recovered from the fruit and identified based on morphological characteristics and molecular sequencing. Fungal characteristics including colony colour, conidia shape, and size, appressorial morphology, presence or absence of perithecia, and setae, and growth rates were used for species identification. Sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region was conducted to confirm the identity of the *Colletotrichum* species isolates. Growth rates and conidial production of the isolates were assessed at 15, 20, 25, and 30^{o}C in 12 hr light: 12 hr dark and constant dark. All three isolates recovered from apple were identified as members of the *C. gloeosporioides* species complex and the two isolates recovered from avocado were identified as members of the *C. acutatum* species complex. For all isolates, the mean colony growth rate and conidial production were significantly higher ($p=<0.05$) when incubated under light: dark conditions than when under constant dark incubation. Temperature significantly affected ($p=<0.001$) the mean

colony growth rate and spore production of the isolates, with the minimal growth rate and spore production of all isolates seen at 15°C and optimum between 25°C and 30°C.

To assess the pathogenicity of *C. acutatum* and *C. gloeosporioides* on *P. vulgaris,* common bean plants were inoculated by spraying with different conidial concentrations (10^2, 10^4, and 10^6 conidia/mL) of one isolate of *C. acutatum* and two isolates of *C. gloeosporioides.* Symptom development on leaves and pods was observed and recorded. All isolates were pathogenic on common bean, *P. vulgaris*, causing significantly (p=0.001) higher disease incidence and severity than the no pathogen controls, but there was no difference in incidence and severity resulting from the different inoculum concentrations. Colonies identified as the inoculating isolates were recovered from the lesions which developed on the bean plants confirming these as the causal agent. This is the first report of an isolate of the *C. acutatum* species complex as a potential pathogen of beans.

To assess the ability of *C. acutatum* and *C. gloeosporioides* to survive on *P. vulgaris* crop debris, artificially inoculated bean plant material was buried in a potting mix: soil mixture at a depth of 5 cm or left on the surface for 6 weeks. The plant material was either coarsely cut or blended prior to inoculation with either *C. acutatum* or *C. gloeosporioides* conidial suspensions. Recovery of the *C. acutatum* and *C. gloeosporioides* from the bean plant tissue was assessed using dilution plating onto agar plates. In all instances, colonies morphologically identified as *C. acutatum* and *C. gloeosporioides* were recovered from the inoculated material incubated on the surface and buried at a depth of 5 cm after six weeks. However, recovery was significantly higher (p=0.001) from blended and buried plant material inoculated with either *C. acutatum* or *C. gloeosporioides* isolates than from the controls. Representative isolates recovered from the tissue were confirmed as members of the *C. gloeosporioides* species complex and the *C. acutatum* species complex by sequencing of the ITS gene region.

Overall, this study has determined that isolates of the *C. gloeosporioides* species complex and of the *C. acutatum* species complex, known to be pathogens of mango, were also able to cause disease in common bean, a crop species commonly grown with mango in Uganda. These two species were also shown to survive for at least 6 weeks on inoculated bean crop debris, and potentially act as a source of inoculum for mango in these cropping systems. For effective control of mango anthracnose disease, strict phytosanitary measures, including avoiding planting of alternative host crops or removal of the crop debris in a mango cropping system should be enforced. This will ensure the removal of potential sources of inoculum for new infections.

Keywords: *Colletotrichum gloeosporiodes*, *Colletotrichum acutatum*, mango anthracnose, *Phaseolus vulgaris*, pathogenicity, plant debris

Acknowledgments

Before all else, I would like to thank my primary supervisor, Prof. Eirian Jones, for her tremendous efforts and patience in guiding me through my Master's journey.
It was a great privilege to have had the chance to experience technical guidance from a person of her caliber. From here I have learned the importance of paying attention to small details. Without her kindness, care, patience, encouragement, and moral support, I would have not been able to complete this work.
I would like to offer credit to my associate supervisor, Prof. Condron Leo for his relentless guidance and indispensable advice during the experiment and writing of this thesis. Particularly, I'm exceedingly thankful to Dean O'Connell for his statistical advice during my analysis stage. To Dr. Seona Casonato, thank you so much for your technical backstopping, moral support, and encouragement, especially at the early start of this project. Even though you could not go through with me to this end, your efforts laid a foundation for this great accomplishment.
Further appreciations go to all staff and students from the department of pest management, Lincoln University, who offered such a warm, friendly, and professional working environment, with special thanks to Alexia, for her sisterly assistance, encouragement, and technical advice throughout this course; to Anish Saha for his support during my molecular work. It's been an absolute pleasure journeying with you all.
To friends at Lincoln, Christchurch, and Lincoln Anglican Parish, St Stephen's Church, In particular, to you Fiona Watson Gould, you provided a home and provided motherly care all through my stay in New Zealand, Sue Gibbs, your persistence during my training outside academic was invaluable. Abigail, you were such a great friend to get along with.
In the most special way my family, which stood with me from the beginning to end. My husband Peter Vivian Acire, you kept your words as a man and supported me despite the distance amidst the stress of taking care of the family single-handedly. No words can express my appreciation for you, may God reward you abundantly and increase your years on earth. To my sons; Destine Kica, Desmond Gen, and Diuk Niye, it was a total sacrifice to walk away from you for this while. You are the reason for this struggle. I owe you my life.
Not forgetting, my mother, thank you for showing me the directions and supporting my earlier education. To my brothers, sisters, and friends back home in Gulu, Uganda, who encouraged me during this academic journey, may God bless you so much.

Table of Contents

List of Tables

List of Figures

Chapter 1. GENERAL INTRODUCTION

1.1 The bean plant

The common bean (*Phaseolus vulgaris),* belongs to the genus *Phaseolus*, subtribe *Phaseolinae*, tribe *Phaseoleae*, subfamily *Papilionoideae* in the family *Fabaceae* (McClean *et al.*, 2008; Singh, 2001). The genus comprises 50 different species of beans including dry beans, green beans, and shelling beans (Delgado-Salinas *et al.*, 1999). *Phaseolus vulgaris* is believed to have originated from the central Mexico (Bitocchi *et al.*, 2012). The crop was independently domesticated in both the Andean and Mesoamerican regions (Bellucci *et al.*, 2014; Bitocchi *et al.*, 2013) and was transported to Africa and Europe in the 1600s (De Ron *et al.*, 2016; Pathania *et al.*, 2014).

Phaseolus vulgaris grows at altitudes ranging from 50 to 3000 m above sea level and can grow in diverse environments. The crop thrives under temperatures ranging from 20 to 28°C (with a minimum of 14^{o}C and a maximum of 32^{o}C). Temperatures above 32^{o}C and below 14^{o}C cause poor pod set resulting in yield loss. Annual precipitation between 400 and 1600 mm per year is ideal for the crop. Soil pH of average 5.8-7.0 is favorable to the crop, a pH less than 5.2 is unsuitable as beans are highly sensitive to acidic soils (Debouck D.G., 1999; Wortmann C.S., 1998).

The global production of *P. vulgaris* in 2016 as dry grain was estimated to be 26.8 million tons, while production as a vegetable bean was 23.5 million tons according to the data of the Food and Agriculture Organization (FAO). The average yields for dry grain and vegetable beans are 913 kg per ha and 1,515 kg per ha, respectively (FAOSTAT, 2021). In Uganda, beans are one of the government's priority food security crops, hence the propensity to plant the common bean, *P. vulgaris* on available land. Production systems are predominantly small-scale (on less than 0.8 ha), accounting for between 60-90% of production with an average production of 0.25 tons (250 kg) per acre. In 2016, the area planted under beans was 683,120 ha with a total production of 1.1 million tons. 'Narobean', 'ROBA', 'K131', 'K132', and 'Nabe' are the common varieties grown in a pure or mixed stand with other crops (Gatsby, 2014).

The plant's parts mostly eaten are the dry seed and fresh (green) pods. Dry or fresh seeds are rich in calories, dietary fiber, starch, dietary protein (18-40% of dry seed weight), minerals, and vitamins (Celmeli *et al.*, 2018a; Kutoš *et al.*, 2003; Osorio-Díaz *et al.*, 2003). The crop offers a major cheap source of protein in the human diet (Broughton *et al.*, 2003). Beans are also rich in unsaturated fatty acids, such as linoleic and oleic acids (Celmeli *et al.*, 2018b) and are thus a major food security crop. In addition, the nitrogen-fixing capacity of the bean crop (> 160 kg of atmospheric nitrogen per ha), through its symbiotic relationship with soil rhizobia, enhances soil fertility and contributes to reducing

environmental pollution resulting from fertilizer application (Beshir *et al.*, 2015; Kamfwa *et al.*, 2019; Rispail *et al.*, 2010).

In spite of the nutritional and economic importance of beans, many kinds of biotic and abiotic stresses affect the bean during its cultivation and storage (Mourice & Tryphone, 2012; Schwartz & Pastor-Corrales, 1994) leading to significant yield and post-harvest losses. Among the biotic factors, bean anthracnose disease caused by several species of *Colletotrichum* is known to result in considerable production losses.

1.2 The pathogen, *Colletotrichum* species

The genus *Colletotrichum* contains several major plant pathogenic species and species complex that cause diseases on a wide variety of woody and herbaceous plants. Although it has a primary tropical and subtropical distribution, it also affects temperate areas (Cannon *et al.*, 2012; Sutton, 1992). *Colletotrichum* species have been included in the 10 most important plant pathogenic fungi in the world, on the basis of their scientific and economic importance (Dean *et al.*, 2012).

The vast majority of *Colletotrichum* species are classified into complexes. Initially, 9 species complexes within the genus *Colletotrichum* were established (Cannon *et al.*, 2012). However, with recent phylogenetic analysis using different gene regions, 11 complexes have been recognised (Doyle *et al.*, 2013a; Marin-Felix *et al*., 2017; Sharma *et al.*, 2013) and 3 more complexes proposed (Damm *et al.*, 2019). The different *Colletotrichum* species complexes are composed of over 189 species with the species within a complex often being widely distributed throughout the world, closely related to each other, and having similar colonization behavior and infection mode (Jayawardena *et al.*, 2016; Sanders & Korsten, 2003).

Colletotrichum species infect a wide host range (Damm *et al.*, 2012; Farr, 2019; Perfect *et al.*, 1999), causing anthracnose disease at pre-harvest and post-harvest stages on a wide range of tropical, subtropical, and temperate fruits, grasses, vegetable crops, and ornamental plants (Crouch *et al.*, 2009; Freeman & Shabi, 1996; Guarnaccia *et al.*, 2017). Several *Colletotrichum* species are also latent plant pathogens, endophytes, or saprobes, which are able to switch to a pathogenic lifestyle when the host plants are subjected to different types of stress, or placed in a postharvest storage (Crous *et al.*, 2016).

1.2.1 Epidemiology of *Colletotrichum* species

The activity of *Colletotrichum* species depends heavily on the environmental conditions, generally, an optimum temperature of 25-28^{0}C favors the growth and sporulation. A pH in the range of 5.8 to 6.5 and high relative humidity (above 95%) promotes conidial germination and appressorium formation (Jeffries, 1990). High moisture encourages the release of conidia from acervuli. On the other hand, environmental conditions such as low or high temperature (below 18^{o}C or greater than 25^{o}C), sunlight, and low humidity rapidly inactivate the pathogens' growth and activity (Ponte, 1996). For infection to occur, *Colletotrichum* species generally need a temperature ranging from 20 to 30°C (Davis *et al.*, 1987). Pathogen dispersal occurs by water splash (overhead irrigation or rain), air currents, insects, infected seeds, and plant debris or other forms of contact (CABI, 2020).

Colletotrichum species persist on and in the host's seed, crop debris, and associated weeds. In healthy plants, *Colletotrichum* species. can survive between seasons as endophytes and as saprobes on dead plant debris (Kumar & Hyde, 2004; Lu *et al.*, 2004). The pathogen can also survive on alternative hosts including decaying wild fruits (Tang *et al.*, 2003). A high carbon dioxide concentration on decomposing plant tissues increases the fecundity (sporulation rate per given lesion) of the pathogen leading to high spread and disease severity (Chakraborty & Datta, 2003). Conidia are also known to survive for 1-2 weeks at a relative humidity as low as 62% and then germinate if placed in 100% relative humidity (Estrada *et al.*, 1992). *Colletotrichum* species are reported to survive for up to 7 months in soil as conidia or mycelium within infected debris under adverse conditions (Pavitra Kumari & Singh, 2017).

1.2.2 Lifestyles of *Colletotrichum* species.

Irrespective of the host plant, the life style patterns of *Colletotrichum* species can be broadly categorized as endophytic, necrotrophic, hemibiotrophic, and latent or quiescent (De Silva *et al.*, 2017a). Of these, the hemibiotrophy lifestyle is the most common (Auyong *et al.*, 2012; Barimani *et al.*, 2013; Perfect *et al.*, 1999). The complexity of the life cycles of many *Colletotrichum* species, their potential to cross infect a wide range of host species, and switch their lifestyle creates major difficulties in managing the diseases they cause (De Silva *et al.*, 2017a; Phoulivong *et al.*, 2012). The lifestyle patterns of the pathogen are highly regulated by specific gene families and biochemical interactions that occur through specific enzymes and secondary metabolites produced at the host-pathogen interface (Gan *et al.*, 2016).

1.2.2.1 Endophytic lifestyle

In this lifestyle, the endophytic fungi (endophytes) may reside within the host plant tissues as symbionts without causing any apparent disease (Hardoim *et al.*, 2015; Vieira *et al.*, 2014). The endophytic interactions between *Colletotrichum* species and the host plant may continuously evolve from mutualistic to parasitic or pathogenic depending on the plant's physiological condition, host genotype, and the environmental conditions (Hardoim *et al.*, 2015; Kogel *et al.*, 2006; Photita *et al.*, 2004; Promputtha *et al.*, 2007).

Many *Colletotrichum* species have been shown to exist as endophytes for part or most of their life cycles in many groups of plants (Cannon *et al.*, 2012; Mahmodi, 2013; Rivera-Orduña *et al.*, 2011; Rojas *et al.*, 2010). The most significant endophytic species of *Colletotrichum* which have been shown to provide beneficial effects to their respective hosts belong to the *C. tofieldiae, gloeosporioides*, *graminicola*, and *boninense* species complexes (Bhagya *et al.*, 2011; Damm *et al.*, 2012; Hiruma *et al.*, 2016; Vieira *et al.*, 2014; Weir *et al.*, 2012c).

Upon colonizing the plant host's roots, a mutualistic relationship develops. 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 (Busby *et al.*, 2016; Hiruma *et al.*, 2016; Kivlin *et al.*, 2013; Porras-Alfaro & Bayman, 2011).

1.2.2.2 Biotrophic and hemibiotrophic lifestyles

Most fungi of the genus *Colletotrichum* do not generally show true biotrophy but a hemibiotrophic lifestyle, where after an initial biotrophic stage of infection the pathogen switches to a necrotrophic lifestyle where it kills the host cells and derives its nutrients from the dead tissue (Münch *et al.*, 2008; Prusky *et al.*, 2013). 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 & Mendgen, 2011). In the biotrophic phase, the pathogen acquires nutrients necessary for its survival from living plant cells, without damaging the host cell.

Symptoms of the disease are not observed at this stage of infection, though the plant's growth can be negatively affected. Fungal biotrophs produce specialized fungal structures, haustoria, that are highly differentiated infection structures necessary for pathogenesis and which facilitate the parasitic relationships with the living host plants to absorb nutrients (Agrios, 2004; De Silva *et al.*, 2017a). The

pathogens secrete a variety of effector proteins that manipulate the host's physiological and biochemical environment mainly by suppressing plant defense responses in order to establish biotrophy in host plants and successfully colonize plant cells (Dou & Zhou, 2012; Gan *et al.*, 2013; Guyon *et al.*, 2014; O'Connell *et al.*, 2012).

1.2.2.3 Necrotrophic lifestyle

Necrotrophic pathogens are those that actively infect and colonize plant cells leading to cell death (van Kan, 2006). Necrotrophy typically secretes 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 (Gan *et al.*, 2013; Kleemann *et al.*, 2012). A necrotrophic life style contrasts with that of biotrophic pathogens which derive nutrients from living cells and therefore must maintain host viability (van Kan, 2006). Nearly all *Colletotrichum* species develop a necrotrophic stage at some point in their life cycles except those few that exist entirely as endophytes (Chang *et al.*, 2014; Prusky *et al.*, 2013).

1.2.2.4 Infection and colonization

Penetration and colonization of host tissues by *Colletotrichum* species generally starts with the germination of conidia and the formation of specialized infection structures, appressoria, which facilitate entry through the host cuticle and epidermal cell walls with the aid of narrow penetration pegs (Figure 1.1) (Perfect *et al.*, 1999; Wharton & Schilder, 2008). Direct penetration through stomata or wounds without the formation of appressoria may also occur (Latunde-Dada *et al.*, 1997; Wharton & Diéguez-Uribeondo, 2004; Zulfiqar *et al.*, 1996). The structure of the host plant surface (cuticle and epidermis thickness, leaf hairs, stomata, and trichomes) is an important factor in the initial infection process as it may act as a barrier against fungal infection (Calo *et al.*, 2006; Serrano *et al.*, 2014).

Post-infection strategies may either be intracellular hemibiotrophy (Figure 1.1A) or subcuticular, intramural necrotrophy (Figure 1.1B) (Münch *et al.*, 2008; O'Connell *et al.*, 2012; Wharton & Diéguez-Uribeondo, 2004). 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 produced enlarged infection vesicles inside epidermal and mesophyll cells. At this stage, the host cells remain alive, and plants do not show any symptoms.

After this initial host colonization, the pathogen shifts to a necrotrophic state and then begins colonizing other plant cells (Wharton & Julian, 1996). In the necrotic phase, thin secondary hyphae grow intracellularly and intercellularly while secreting cell wall degrading enzymes or toxins that kill the host

cells (O'Connell *et al.*, 2012). The duration of the transition period between the biotrophic and necrotrophic phases varies among species of *Colletotrichum* and depends on the host and environmental conditions (Crouch *et al.*, 2009; Peres *et al.*, 2005; Ranathunge *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. The pathogen subsequently survives on the contents of dead or dying cells to complete its life cycle (Gan *et al.*, 2013; Kleemann *et al.*, 2012; Stone *et al.*, 2001). As the fungal growth progresses in plant tissue, the most characteristic symptom of the disease, necrosis of infected tissues, appears.

In common bean, depending on the variety, typical symptoms on pods are reddish-brown to black circular spots that develop into light-colored cankers surrounded by a dark brown to black border (Schwartz & Pastor-Corrales, 2005). As the disease advanced, wilting and flagging of chlorotic leaves often occurs on the seedling plants, similar to that of other foliar pathogens. Severely infected premature pods abort and fall early, while pods that mature produce infected seed with dark cankers that make the seed unmarketable to consumers (Pastor Corrales & Tu, 1989).

Lesions caused by *Colletotrichum* species infection on mango occurs on leaves, petioles, flower clusters (panicles), and fruits. Infected leaves develop small, angular, brown to black lesions on both surfaces which start as small spots that enlarge to form extensive dead areas resulting in defoliation in dry weather (Ploetz *et al.*, 1996). On the panicles, small, dark-brown, or black spots develop which enlarge, coalesce, and kill the flowers before fruit development, thus significantly reducing yield. The lesions which develop on petioles, twigs, and stems are like the ones on fruits, leaves, and flowers being black expanding lesions. Infected ripe fruits develop sunken, prominent, round dark brown to black decay spots before or after harvest. The spots that form on the fruit can coalesce and eventually penetrate deep into the affected fruits resulting in extensive rotting. Early infection can cause premature fruit drop or may persist leading to latent infection as the pathogen remains dormant until the ripening stage (Arauz, 2000).

Figure 1. 1 Post-infection strategies adopted by *Colletotrichum* species: A) intracellular hemibiotrophy B) subcuticular, intramural necrotrophy C) localized hemibiotrophic infection with multilobed primary hyphae, and D) extended hemibiotrophy. C) conidia, AP) appressoria E) epidermal cells, M) mesophyll cells, PH) primary hyphae, SE) secondary hyphae, MLPH) multilobed primary hyphae. From (De Silva *et al.*, 2017b)

To complete the cycle of infection, *Colletotrichum* species may either undergo asexual/anamorph or sexual/teleomorph reproductive stages which occur on the host plant or in plant debris (Figure.1.2) (Sutton, 1992). In the asexual stage, Colletotrichum produces an acervulus. In the plant tissue, the acervuli are sub-cuticular (Khan & Hsiang, 2003). With the growth of a stroma, formed by the fungal mycelium between the plant epidermis and cuticle, the tension on the cuticle increases, until the mature acervulus breaks the cuticle and is exposed to the environment. Then, from the stroma present in the acervulus, conidiophores are initiated, from which unicellular conidia of the pathogen are produced (Curry *et al.*, 2002). The conidia are produced in a water-soluble mucilaginous matrix, composed of glycoproteins and germination inhibitors, which protect the conidia against desiccation and toxic molecules produced by the host (Leite & Nicholson, 1992). Upon contact with water from raindrops or irrigation, and assisted by the wind, conidia produced in acervuli are spread to new locations on the same host plant or to new hosts, which may lead to a new cycle of infection (Ntahimpera *et al.*, 1997).

If the sexual stage is present, the pathogen sexually reproduces dark long-necked perithecia containing ascospores inside asci. Perithecia can act also as survival structures that help pathogens to overwinter or survive periods without a susceptible host. Eventually, if conditions are favorable for the pathogen, the ascospores are released from the perithecia, which, when released, may be able to infect neighboring plant tissues in which they are deposited (Sutton & Shane, 1983). 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 the development of the sexual stage to restart the life cycle. While the asexual stage is most associated with disease symptoms, the sexual stage of *Colletotrichum* is rarely observed in many important species complexes and is poorly understood (Barcelos *et al.*, 2014a; Cannon *et al.*, 2012; Damm *et al.*, 2012; Ishikawa *et al.*, 2010). However, perithecia are readily produced in cultures of species in the *gloeosporioides*, *boninense*, *destructivum,* and *graminicola* complexes (Ishikawa *et al.*, 2010; Manandhar *et al.*, 1986; Rodríguez-Guerra *et al.*, 2005; Vaillancourt *et al.*, 2000).

1.2.2.5 Quiescent lifestyle

This is an extended period of time in the fungal life cycle in which the pathogen becomes dormant within the host after colonization before it switches to an active phase, often after harvest, storage, transportation, and sale of produce (Prusky *et al.*, 2013; Ranathunge *et al.*, 2012). During quiescence, pathogen activity appears to be suspended and almost no growth occurs. This lifestyle phase is common in *Colletotrichum* species that cause postharvest disease in fresh fruits and vegetables (Gomes *et al.*, 2013; Shaw *et al.*, 2016; Van Kan *et al.*, 2014). Both *C. gloeosporioides* (Prusky, 1996) and *C. acutatum* (Adaskaveg & Hartin, 1997) are known to have a quiescent period in their life style before causing postharvest disease of fruits.

The 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 lifestyle (Prusky *et al.*, 2013). A detailed study of *C. acutatum* and *C. gloeosporioides* infection on olives showed that in the quiescent period, the conidia of the pathogen germinate, form appressoria, and then remain dormant on non-targeted vegetative organs such as leaves and branches, which served as a source of overwintering inoculum. Once environmental conditions became favorable, infection developed and spores are dispersed via wind or rain to all plant organs (Talhinhas *et al.*, 2011).

Figure 1. 2 Disease cycle of *Colletotrichum* species from (De Silva *et al.*, 2017a).

1.3 Identification of *C. acutatum* and *C. gloeosporiodes* species

Traditionally, *Colletotrichum* species from different hosts have been differentiated based on morphological and cultural characteristics (Sutton, 1992). However, the influence of environmental factors on the stability of morphological and cultural features (Hyde *et al.*, 2009; Weir *et al.*, 2012c), the recognition of species complexes and intermediate forms, and the lack of standardization of the cultural conditions used in the different studies makes the morphological identification problematic. For

example, the *C. gloeosporioides* and *C. acutatum* species complexes consist of 22 species plus one subspecies, and 31 species respectively (Damm *et al.*, 2012; Weir *et al.*, 2012a) makes it very difficult to separate the species based on distinguishing morphological characteristics. Despite the limitations posed by this method, the use of morphological structures (conidia and appressoria morphology, development of perithecia with asci and ascospores), cultural (colony colour and colour variations), and physiological (mycelial growth rate) (Damm *et al.*, 2012; Weir *et al.*, 2012c) still remain key features used to distinguish species of *Colletotrichum* (Hu *et al.*, 2015; Hyde *et al.*, 2009), especially in the absence of more advanced methods such as molecular sequencing.

Findings from previous studies show marked variability and to some extent consistencies in morphological and cultural features of *C. acutatum* and *C. gloeosporioides* species complexes. Hassan *et al.* (2018) reported wide variability o among colonies of isolates of *C. gloeosporioides* complex, with colonies being white, black, or grey in colour. The conidia of species within the *C. gloeosporioides* complex ranged from 12.0 to 18.6 µm long and 3.5 to 6.1 µm wide (Hassan *et al.*, 2018), with similar dimensions (10-16 µm in length and 1-13 µm in width) and the mean growth rates (11.2-12 mm/day at 25^{o}C) to those reported by Weir *et al.* (2012c). Zhang *et al.* (2021) reported results consistent with those of Hassan *et al.* (2018) and Weir et al., 2012b, where the dimensions of conidia of *C. gloeosporioides* were 10.2-16.1 µm in length and 4.2-7.3 µm in width. Their results, however, varied in the mean colony growth rate, 10.4-10.8 mm/day of the species. The conidia of *C. gloeosporioides* isolates were reported by Xie *et al.* (2010) to be oblong with obtuse ends. Colonies showed a dense, white mycelial growth which turned to a dark olive-grey colour. Setae of *C. gloeosporioides* were dark brown and gradually tapered along their entire length to their apices. In the *C. acutatum* species complex, white to pink mycelia, and pink/salmon colony colour on the reverse side were observed. Conidia of *C. acutatum* measured 8-15 µm in length and from 1-4 µm in width, with the mean growth rate of 12-15 mm per day at temperature 25^{o}C.

In terms of shape and size, Xie *et al.* (2010) reported that the conidia within the *C. acutatum* complex were elliptical to fusiform in shape, being 11.2-15.5 µm in length and 4.1-4.7 µm in width. The colony colour of *C. acutatum* isolates was white to grey, brown. The setae of *C. acutatum* were brown to dark brown, tapered, and generally aseptate. In contrast, Damm *et al.* (2012) showed that the major features of *C. acutatum* differed from those reported by Xie *et al.* (2010) whereby the conidia of *C. acutatum* were straight, cylindrical to clavate with one end round and one end slightly acute or both ends round in shape. These were reported to be 15.5-20.5 µm in length and 4.5-5 µm in width. The colonies were

reported to produce white to pink mycelium, producing salmon, orange to olivaceous grey acervuli, with the reverse being pink/salmon in colour.

Regarding colony growth, Kumara and Rawal (2010) found that isolates of *C. gloeosporioides* grew fastest at 28^{o}C, followed by 30^{o}C, and 25^{o}C. Incubation at a temperature of 15^{o}C was reported to inhibit colony growth. Sporulation at different temperatures followed a similar trend to that of mycelia growth. Similar results were reported by Sangeetha and Rawal (2010) where the maximum mean colony diameter was at 28°C, followed by 25°C. Incubation at 15°C resulted in the least growth of all the isolates tested. Overall, temperatures in the range of 25-28°C supported the optimum growth of all isolates of the *C. gloeosporioides* complex. Sporulation of all isolates was found to correlate positively with colony growth at temperatures 25^{o}C and 28^{o}C. There was limited to no spore production at 15, 20, and 30°C. In contrast, Khanzada *et al.* (2018) reported *C. gloeosporioides* to grow fastest at 30°C, followed by 35^{o}C, 25°C, and then 20°C. No growth was recorded at incubation temperatures of 15°C, 40°C, and 45°C.

Kenny *et al.* (2012) reported isolates of *C. acutatum* to grow fastest (6.3 mm/day) at 21°C, while *C. gloeosporioides* attained maximum growth (15.5 mm/day) at 25-31°C. Temperatures < 9^{o}C and >35^{o}C did not favour colony growth of either species. Morkeliūnė *et al.* (2021) reported that the optimal growth of *C. acutatum* isolates incubated under dark was in the temperature range of 20-25^{o}C, with the lowest at 5^{o}C. A higher colony growth was achieved in isolates incubated under equal hours of light and dark (12 hr light 12 hr dark) than under continuous light.

Soltani *et al.* (2014) reported that for *C. gloeosporioides,* the mean mycelial growth rate was similar when incubated under constant dark and under continuous light at 28 to 30°C. However, sporulation was higher on all of the media tested when the cultures were incubated under continuous light, with only very low numbers of conidia produced under continuous dark incubation. Further, incubation under a 16/8 hr light/darkness regime resulted in the highest spore production. Culture medium was also shown to affect *C. gloeosporioides* sporulation, indicating that *C. gloeosporioides* sporulation is highly dependent on light and culture media. Khanzada *et al.* (2018) reported higher colony growth when *C. gloeosporioides* isolates were incubated under 12/12 light/dark than under all other light conditions including constant light, 16/8 light/dark, and 8/16 light/dark regimes, but not incubation under constant dark.

Due to the variability in the cultural and morphological characteristics of isolates within a species as well as the overlap between different species within a complex, molecular identification methods have become increasingly important for the identification of *Colletotrichum* species The utilization of available DNA sequences of various genomic regions has enabled more precise identification of species within the genus *Colletotrichum,* including the species complexes (Weir *et al.*, 2012a). Most *Colletotrichum* species can be identified based on sequences of the nuclear ribosomal internal transcribed spacer (ITS) region or a combination of ITS and other gene regions including actin (ACT), calmodulin (CAL), chitin synthase (CHS-1), and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Diao *et al.*, 2017; Liu *et al.*, 2016; Weir *et al.*, 2012c). Advanced bioinformatics tools have allowed the design of species-specific primers for accurate detection of *Colletotrichum* species (Kamle *et al.*, 2013) using nested PCR, initially with genus-specific PCR followed by species-specific PCR were able to clearly discriminate *C. gloeosporioides* from other species. Similarly, Sanabria *et al.* (2010) reported the *C. gloeosporioides* specific primer, CgInt/ITS4, and *C. acutatum* specific primer, CaInt2/ITS4, was able to clearly discriminate these two species.

1.4 Pathogenicity of *Colletotrichum* species on common bean, *Phaseolus vulgaris.*

Several species of *Colletotrichum* are reported as a major cause of anthracnose disease in numerous key annual crops around the world (Sharma & Kulshrestha, 2015). Anthracnose disease of the bean, caused by *C. lindemuthianum* is most serious under cool and humid environments where it may cause up to 100 percent yield loss (Padder *et al.*, 2007; Sharma *et al.*, 2008). The pathogen, *C. lindemuthianum*, primarily invades injured or weakened plant tissues. There are only a few reports available that indicate that other species of *Colletotrichum* also cause anthracnose in the common bean. However, several species of *Colletotrichum* including *C. falcatum, C. gloeosporioides, C. truncatum, C. sansevieriae, C. acutatum, C. capsici,* and *C. coccodes* are reported to cause damage on a wide range of plants (Gautam, 2014). For example, *C. gloeosporioides*, a major pathogen of mango anthracnose disease, is known to also be seed-borne in some bean cultivars and found to be pathogenic to beans at the seedling stage (Marcenaro & Valkonen, 2016). *Colletotrichum gloeosporioides* is also reported to cause cankers and severe wilting when inoculated onto lima bean and the cultivar 'INTA Rojo' at the seedling stage under a controlled environment (Chakraborty, 2019; Marcenaro & Valkonen, 2016). Barcelos *et al.* (2014b) reported only mild symptoms to develop on the leaves and pods of common bean plants following artificial inoculation with conidia of *C. gloeosporioides* species complex isolates, but inoculation with *C. magna* isolates resulted in no symptoms. Chakraborty *et al.* (2019a) further reported the infectivity of *C.*

gloeosporioides on the common bean. In their study, typical disease symptoms developed on *P. vulgaris* leaves inoculated with *C. gloeosporioides* conidial suspensions two days after inoculation. Microscopic examination of the inoculated leaves indicated the formation of the appressoria and acervuli on the leaf surface. Mahmodi (2013) studied the pathogenicity of *C. gloeosporioides* on other legume crops such as cowpea, pigeon pea, soybean, peanut, and lentil other than common bean, with *C. gloeosporioides* shown to be infective on cowpea and soybean. In addition, the ability of many *Colletotrichum* species to survive on a broad host range and develop unique relationships with particular hosts to cause asymptomatic infections presents an important question regarding the epidemiology of these pathogens in a mango cropping system. The infection of a particular host (e.g. bean) by one or more *Colletotrichum* species may serve as an inoculum source for infecting other plant species (Phoulivong *et al.*, 2012; Udayanga *et al.*, 2013). Thus *P. vulgaris,* being a key crop commonly alternated with other crops in a mango cropping system, could potentially act as a reservoir of *Colletotrichum* species inoculum that can cause anthracnose disease of mango.

1.5 Survival of *C. acutatum* and *C. gloeosporioides* on plant debris and in soil.

Parikka and Lemmetty (2010) tested the ability of *C. acutatum* to survive on the debris of several weed species in the glasshouse and reported that the pathogen was able to survive in the weed's residues and to overwinter in living plants for up to one year. In Nordic conditions, *C. acutatum* was found to survive in soil or on the soil surface in artificially infected strawberry (*Fragaria* x *ananassa*) residue for nearly 3 years (Parikka *et al.*, 2014). *Colletotrichum acutatum* is also known to survive on dead wood, mature and mummified berries of grapevines (*Vitis vinifera*) from the previous year, and to act as potential sources of inoculum for new infections (Samuelian *et al.*, 2012). Conidia surviving on plant material on the ground may be dispersed via wind and rainwater splash to infect new grapevine tissues (Samuelian *et al.*, 2012). A study in Brazil reported the survival of *C. acutatum* and *C. gloeosporioides* on apples (*Malus domestica*) during winter in dormant buds, on dormant twigs, and on fallen leaves (Hamada & De Mio, 2017). Under field conditions, conidia of *C. acutatum* were detectable up to 12 months after the incorporation of infected Leatherleaf Fern (*Rumohra adiantiformis*) leaf debris into the soil (Norman & Strandberg, 1997). The survival of *C. acutatum* in soil, infected pepper (*Capsicum annuum*) fruit, and fruit debris incorporated into the soil at different temperatures under different soil moisture levels was also investigated by Kang *et al.* (2009). Conidia of *C. acutatum* were found to survive in both sterile and non-sterile soil at 4 and 10℃ for 18 weeks. On completely dried infected pepper fruits, *C. acutatum* survived for 18 weeks at temperatures from 4 to 20°C. These findings suggest that soil temperatures and moisture affect the survival of *C. acutatum* in infected plant debris incorporated into the soil.

Colletotrichum gloeosporioides was reported to survive for more than seven months in soil as conidia or mycelium in naturally infected mango debris under adverse conditions (Kumari *et al.*, 2017). The survival of *C. gloeosporioides* was observed to generally reduce with increasing soil depth and duration of burial of the infected plant materials, indicating its long survival especially close to the soil surface. At a depth of 5 cm, survival of *C. gloeosporioides* was shown to decrease from 81.6% after 1 month to 0% after 7 months (Kumari *et al.*, 2017). Colonies of *C. gloeosporioides* have also been shown to remain viable for up to 90 days when infected black pepper (*Piper nigrum*) plant parts were buried in the soil, and up to 150 days in plant materials stored in paper bags (Kumari, 2002). In a mango cropping system, Takushi (2015) found conidial masses of *C. gloeosporioides* to survive on mango leaf debris for more than 10 months under favourable moisture and temperature conditions in the field. This result suggests that infected debris in mango fields may serve as an infection source for subsequent mango anthracnose. Nonetheless, the low survival of *C. gloeosporioides* in soils and decaying plant pieces has also been reported. Ripoche *et al.* (2008) gave an account of the viability of the pathogen on artificially inoculated leaf and stem residues of yam placed on the soil surface or buried at 0.1 m depth decreases with duration. The number of viable *C. gloeosporioides* conidia decreased sharply after the first month. However, *C. gloeosporioides* recovery was higher from the residues placed on the soil surface due to a larger amount of readily available substrates compared with the buried material which decomposed readily and where the survival of *C. gloeosporioides* was limited by competition from microorganisms decomposing the residues. This finding demonstrates the inability of *C. gloeosporioides* to survive in the soil for a longer period, unlike on non-decomposed plant residues. The survival of *C. gloeosporioides* in soil was further assessed by Ekefan *et al.* (2000), who showed that *C. gloeosporioides* was able to survive for over 20 weeks in sterile soil and only 8 weeks in non-sterile soil. These authors suggested that interactions between *C. gloeosporioides* and other soil micro-organisms may influence the duration of *C. gloeosporioides* survival in the soil.

1.6 Mango production and cropping system in Uganda

In Uganda, mango is by far the most common fruit grown across the country as it is adapted to almost all the agro-ecological zones of the country, dry or humid, montane and lake shorelines UIA (2010). Cultivation of local varieties is mainly concentrated in the relatively hot and dry Northern, Northeastern, and West Nile zones, while the exotic cultivars such as ‘Boribor’, ‘Tummy’, ‘Kate’, ‘Kent’, and ‘Alfonso’ are mostly grown in the Central and Western regions of the country. The exotic varieties are of superior quality to the local varieties as they have higher juice yields, good flavor, are less fibrous; and are more appealing to both the local and the international markets (Okoth *et al.*, 2013). The mango industry is

continuing to grow and there is a desire for the fruit to contribute substantially to the local economy, with the government making considerable efforts to promote the production.

Mango cultivation is mainly carried out under a mixed cropping system. Production ranges from small (≥ 0.4 ha), medium (0.88-2.04 ha) to large scale (> 2.4 ha). Because of limited care given to the growing trees, the plants take between 4 and 8 years to begin to bear fruits. To make the most use of the available land, most growers also grow other crops underneath the mango (Figure 1.3). These crops are mainly covered crops such as beans, soybean, groundnuts, vegetables, cowpea, and green grams, and this continues up to the time when the mango plants begin to produce large canopies. During the harvesting of these alternate crops, the debris is left in the orchard and later used as a mulch or ploughed back into the soil in the subsequent season.

Many diseases affect mango production in Uganda, however, mango anthracnose caused by *Colletotrichum gloeosporioides* is the most prevalent (Nelson, 2008b). *Colletotrichum acutatum* has also been reported to cause infection in some fields (Freeman *et al.*, 2008; Jayasinghe & Fernando, 2009). In most mango orchards, the incidence of *C. gloeosporioides* can range from 32-72% on leaves and from 36-100% in flowers and fruits produced under wet or very humid conditions (Arauz *et al.*, 1994; Sanders *et al.*, 2000). Though not quantified, *C. gloeosporioides* causes huge economic losses to farmers owing to its unique etiology, mode of infection, and stage of plant development it attacks. The postharvest phase is the most economically significant as it drastically affects the fruit's quality. Chemical control methods have been utilized to control the disease, though cultural methods are still widespread because of the high cost of chemicals. The most common cultural methods used include botanical sprays, ash sprays, filed sanitation, and fruit bagging.

Figure removed due to copyright

Figure 1. 3 Bean plants growing alongside mango during the early stage of orchard establishment. Field photo, Crop sector, Omoro District Local Government, Uganda, 2021.

1.7 Statement of the problem

In Uganda, based on the available information, climatic and other favorable soil conditions appear to support the survival of *Colletotrichum* species in susceptible host plants and other alternate crop debris throughout the year. The inoculum for the initiation of mango anthracnose disease is assumed to come from sources within production fields as soil fumigation is not a common practice among fruit growers. Additionally, adherence to phytosanitary measures during mango seedling production, distribution, and transplanting is often lacking. Since infected transplants could also act as potential sources of the inoculum, nurseries providing transplants could be an important contributor to the introduction of pathogens into production fields. Most importantly, the integration of mango plants, especially in the early stage of orchard establishment, with other crops such as bean, groundnut, maize, soybean, citrus, avocado, banana, coffee, and pawpaw, and overall poor weed and debris management both within the orchards and surrounding areas, could present a high risk for anthracnose disease development. Since species within the *C. gloeosporioides* complex are known to cause infection on a broad range of host plants (Mordue, 1971), cross-infection could occur between the crops inter-planted with the mango. Species within the *C. acutatum* complex are also reported as a pathogen of mango (Jayasinghe & Fernando, 2009; Mo *et al.*, 2018) and are also reported to have a wide host range (Wharton & Diéguez-Uribeondo, 2004). The presence of such alternate plant species growing in the vicinity of mango fields in Uganda could thus play a pertinent role in the epidemiology of the disease by acting as potential sources of inoculum. Despite a significant gain in knowledge of the host range of both *C. acutatum* and *C. gloeosporioides*, there is limited knowledge on the potential role of alternate host species to drive the spread of the pathogen in a mango cropping system. Field assessment indicates that severely infected mango plants with typical anthracnose symptoms are mainly observed in intercropped and poorly managed fields (Acema *et al.*, 2016). Additionally, many alternate host species are asymptomatic to anthracnose disease due to the endophytic nature of *Colletotrichum* species, and as such, the infection likelihood of mango due to under-planting of green crops may be underestimated. There are many alternate plant species that are grown in a mango cropping system in Uganda and are known to be susceptible to *Colletotrichum* species However, this study investigated the pathogenicity of isolates within two *Colletotrichum* species complexes, *C. gloeosporioides,* and *C. acutatum*, on the common host, *P. vulgaris,* that may be contributing to mango infection in Uganda.

1.8 Research hypothesis

1. The under-planted annual crop, *P. vulgaris,* used in mango orchards in Uganda, is a host for *Colletotrichum* species

2. *Colletotrichum* species remain viable in *P. vulgaris* crop debris, either on the soil surface or buried, for more than 6 weeks.

1.9 Objectives of the study

1. Identification of *Colletotrichum* species isolates obtained from infected plant materials using morphological characteristics and molecular sequencing.

2. Assessment of the pathogenicity of isolates of *C. acutatum* and *C. gloeosporioides* species complexes on *P. vulgaris* under a glasshouse condition.

3. Determine the survival of *C. acutatum* and *C. gloeosporioides* species complexes on *P. vulgaris* debris either buried or left on the soil surface.

Chapter 2. Identification of isolates of *Colletotrichum acutatum* and C. *gloeosporioides* obtained from infected plant material

2.1 Introduction

Colletotrichum is a cosmopolitan fungal genus comprising more than 188 species, with 164 species distributed among 14 species complexes (Guarnaccia *et al.*, 2017; Marin-Felix *et al.*, 2017). *Colletotrichum gloeosporioides* and *C. acutatum* species complexes which are composed of at least 22 and 30 species, respectively (Damm *et al.*, 2012; Weir *et al.*, 2012a) have been associated with anthracnose diseases worldwide (Damm *et al.*, 2012; de Silva *et al.*, 2019b; Douanla-Meli & Unger, 2017; Guarnaccia *et al.*, 2017; Huang *et al.*, 2013; Yin *et al.*, 2017). The pathogen affects crops both in the field and at postharvest stages. Although these two species complexes are associated with the same fungal genus, differences in their pathogenicity, morphology and cultural characteristics have been reported.

Accurate identification of the *Colletotrichum* species causing anthracnose is crucial as this is required to inform the best control strategies for that specific anthracnose disease (de Silva *et al.*, 2019a). Identification of *Colletotrichum* to species level was traditionally reliant on host, cultural and morphological characters. However, the approach is constrained by similarity in symptoms caused by, and morphological and growth characteristics of, species within the genus, as well as variation in the response of isolate with a species to environmental factors (Adaskaveg & Hartin, 1997; González *et al.*, 2006b). Most importantly, species within a complex may share a common morphological characteristic, such as conidial shape and size, colony color, spore production, and growth rate which makes it very difficult to identify species based on morphology alone. Thus, molecular approaches have been widely used to precisely identify species of *Colletotrichum*. Combinations of morphological and molecular approaches have been reported to yield reliable results for *Colletotrichum* species delineation (Freeman *et al.*, 1998; Gunnell & Gubler, 1992; Sreenivasaprasad & Talhinhas, 2005). Conventional PCR allows amplification of the target region of fungal DNA for the species identification (Jones *et al.*, 2011; Sreenivasaprasad & Talhinhas, 2005). The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) has been widely targeted and used as the universal barcode for fungi.

Since the growth of different *Colletotrichum* species is greatly influenced by light and temperature, and other nutritional factors such as the nitrogen and carbon sources, and pH of the substrate, species identification based on a combination of morphological and molecular approaches is therefore

important for accurate differentiation. The purpose of this study was to characterize and confirm the identity of *Colletotrichum* species isolates obtained from plant materials using cultural and molecular means.

2.2 Materials and methods

2.2.1 Fungal isolation from infected tissues

Five naturally infected fruit samples (three apples and two avocados) showing anthracnose symptoms were obtained from a local fruit shop in Prebbleton, New Zealand. The fruits used were all grown in New Zealand. Avocado and apple fruits were chosen because they are host to *C. gloeosporioides* and *C. acutatum*. Secondly, these plants are commonly intercropped with beans in Uganda. All fruit were washed with tap water and surface sterilized in a 0.5% sodium hypochlorite (NaOCl) solution for 45 s and then rinsed with sterile water. The samples were then dried on paper towels in a sterile airflow in a laminar flow hood and again disinfected with 70% alcohol solution by dipping.

Tissue pieces (10) were cut from the edge of lesions from each infected avocado and apple fruit using a sterile scalpel and placed individually in the centre of the Petri dish containing potato dextrose agar (PDA; Difco Laboratories, MI, USA) amended with streptomycin (5 mg /mL; Sigma Aldrich) and penicillin (5 mg /mL; Sigma Aldrich). In addition, any perithecia that were observed microscopically on the surface of the infected fruits were detached and crushed using sterile forceps and placed in the centre of a Petri dish containing PDA amended with streptomycin and penicillin. The samples were incubated at 25^{o}C under a 12 hr photoperiod for 14 days. Any colonies showing characteristics of *Colletotrichum* species growing from the tissue pieces were subcultured onto fresh PDA plates. The plates were incubated at 25^{o}C under a 12 hr photoperiod for 7 days. One isolate was selected from each fruit since all samples obtained a particular fruit exhibited uniform colony colour. Isolates C.g1, C.g2, and C.g3 were recovered from apple fruit and isolates C.a4 and C.a5 from avocado, making a total of 5 isolates.

To obtain pure cultures, the fungal isolates were sub-cultured by hyphal tipping. The tip of an actively growing hypha was removed using a hypodermic needle, under a stereomicroscope, placed directly onto the centre of a PDA plate, and incubated at 25^{o}C in a 12 hr photoperiod for 14 days. The pure culture isolates were stored as mycelial discs and spore suspensions in 1.5 mL Eppendorf tubes containing 600 µL of preservation media (60% glycerol, 20% glucose, 20% peptone (Pancreatic casein, Sigma-Aldrich®), and 10% yeast extract). Two tubes were prepared per isolate and stored at -80^{o}C.

2.2.2 Morphological identification

The grouping of *Colletotrichum* isolates to morphotypes was based on 9 characteristics including colony colour, conidial size and shape, conidiomata, appressoria shape, presence or absence of perithecia, presence or absence of setae, colony growth rate and pattern on PDA, and sporulation capacity and used for preliminary species identification as per a published fungal key (Freeman *et al.*, 2008; Nagamani, 2009). For each isolate, the stored cultures were used to inoculate PDA plates. Fifteen PDA plates were prepared for each isolate; 3 for each morphological assessment (i.e. 3 for colony colour, 3 for conidial shape and size, 3 for peritheica and conidiomata production, 3 for appressoria production, and, 3 for setae production), except for colony growth and spore production. The plates were incubated at 25^{o}C in 12: 12 hr photoperiod for specified periods depending on the cultural characteristics being assessed.

To assess the colony colour, three replicate plates per isolate were randomly selected after 5 days of growth. Colonies were directly observed under natural daylight. The color of the growing mycelia on both the upper and reverse side of the colony was described using colour charts (Rayner, 1970).

The size and shape of conidia were examined and recorded from 14-day-old colonies growing on PDA using a slide culture technique upon staining a small portion of the culture previously picked with a hypodermic needle with a drop (0.2 µL) of lactophenol cotton blue and a drop of lactic acid (Appendix A, Table A.1). All slides were then covered with a coverslip and observed under a compound microscope at X400 to determine the conidial shape and size. For each isolate, 5 conidia were randomly selected for assessment and placed in one of three shape categories: (1) conidia cylindrical with straight sides and pointed at one end, (2) conidia cylindrical with straight sides and rounded at both ends, and (3) oblong conidia.

The presence or absence of perithecia and conidiophores were directly observed from 21 day-old cultures under a stereomicroscope at X50. To examine the shape of appressoria, appressoria were induced using the slide culture technique as described by Johnston and Jones (1997). Briefly, a small square (approx 10 mm) of PDA was inoculated on one side with conidia taken from each of the sporulating isolates and immediately covered with a sterile cover slip (15 mm x 15 mm). After 21 days of incubation at 25^{o}C, the cover slip was removed, placed in a 0.2 µL drop of lactic acid on a glass slide, and the morphology of the appressoria which developed on the cover slip was observed under a compound microscope at X400.

To assess the presence or absence of setae, for each isolate, the perithecia from a 21-day-old culture were crushed on a glass slide and observed under a compound microscope at X400 after staining with lactophenol blue and clear lactic acid. The presence or absence of setae was recorded.

2.2.3 Assessment of colony growth and spore production by isolates under different temperatures and light regimes

2.2.3.1 Physiological assessment of appropriate media for isolate growth

To assess the most suitable media to support both colony growth and spore production of isolates under different temperature and light regimes, four media were prepared; full and half strength PDA, Malt Extract Agar (MEA; Difco™, USA), and 10% water agar 10% (WA) were prepared (Appendix A, Table A.2). Petri dishes (90 mm diameter) were filled with approximately 20 mL agar. Each of the five isolates was subcultured onto each of the four growth media and the plates were sealed with parafilm. The plates were incubated at 20 or 25°C in a 12 hr photoperiod for 10 days. For each isolate, three replicates were set up for each treatment (media x temperature) and the plates for each temperature arranged in a randomized block design in one of the two incubators, each incubator representing one of the incubation temperatures. The diameter of the colonies (mm) was recorded daily in two perpendicular directions using a digital caliper (Mitutoyo, Kanagawa, Japan) until the colony reached the edge of the plate. The number of spores/mL was counted using a hemocytometer after 7 days of incubation. Briefly, a single plug (2 mm diameter) was taken from the centre of the isolate colony next to the original central inoculation plug and placed into a 1.7 mL Eppendorf tube containing 1000 µL of Milli-Q. The tube was vortexed for approximately 5 s, and the conidial concentration in the resulting spore suspension was determined using a hemocytometer. The media that supported the fastest colony growth and higher spore count was selected for the growth and sporulation assessment.

3.2.3.2 Colony growth

Malt Extract Agar was used as it was shown to support the fastest mycelial growth rate for all 5 isolates and support the greatest sporulation. Fresh MEA plates were inoculated with each of the 5 isolates taken from the previously-stored pure cultures and the inoculated plates were incubated at 5 different temperatures (15°C, 20°C, 25°C, and 30°C) with one set of plates for each temperature incubated in 12 hr light: 12 hr dark and another set in the continuous dark. For each isolate, 10 replicate plates were set up for each temperature/light treatment with separate incubators used for each temperature and light treatment. The plates were allocated within each incubator in a randomized block design, with one shelf representing a block. The cultures were grown for 14 days. To determine colony growth, the diameter of

each colony in two perpendicular directions were measured using a digital caliper. Measurements (mm) were recorded after every 48 hr (7 measurements per replicate) and continued up to 336 hr (14 days) for each treatment. The mean growth (mm) was calculated at each assessment time.

3.2.3.3 Sporulation capacity

The number of conidia produced was determined by hemocytometer counts after 192 hr and 336 hr incubation. Conidia were counted from the same plates used for growth measurements. For the 192 hr assessment, disc samples were taken from each plate, with the plates then returned to the incubator for further assessment at 336 hr. To count the conidia, a single plug (2 mm diameter) was taken from the centre of the isolate colony next to the original central inoculation plug and placed into a 1.7 mL Eppendorf tube containing 1000 µL of Milli-Q. The tube was vortexed for approximately 5 s, and the conidial concentration in the resulting spore suspension was determined using a hemocytometer (Alves & Moraes, 1998). Ten replicate plates were assessed for each isolate and temperature/light treatment.

2.2.4 Molecular sequencing for species identification

The isolates were identified to species level by sequencing the ITS regions using universal primer pairs ITS1 and ITS4.

2.2.4.1 DNA extraction

Each of the isolates, C.g1, C.g2, C.g3, C.a4, and C.a5, were inoculated onto PDA and grown for 3 days at 25^{o}C in a light/dark regime. For DNA extraction a small amount of mycelium was scraped from the surface of the cultures using sterile pipette tips and placed into 1.7 mL Eppendorf tubes. DNA of each fungal isolate was extracted using the Chelex® resin methods as outlined by (Henderson *et al.* (2019). Briefly, approximately 250 µL of 10% Chelex-100 (Chelex®100 resin, 50-100 mesh, sodium form; Bio-Rad, Richmond, CA) was added into each of the 1.7 mL Eppendorf tubes containing the mycelial samples of each of the isolates. The mycelia were vortexed for 3 s in the solution and the tubes placed on a heat block at 100^{o}C for 10 min, then removed and vortexed for 3 s more then placed onto the heat block for more 5 min. The tubes were then centrifuged for 10 min at 13,000 rpm. Aliquots of the supernatant (150-200 µL) were transferred into new sterile Eppendorf tubes. One extraction per isolate was undertaken. Each sample's amount and quality of DNA were assessed based on an absorbance ratio of 1.80–1.90 at 260/280 and recorded using a Nanodrop spectrophotometer. DNA concentration was adjusted to 10-60 ng/µL. Aliquots of the samples were stored at −20°C until used for amplification by PCR.

2.2.4.2 PCR amplification

The ITS region of the selected fungal isolates was amplified using the universal primers ITS1 (5' -TCCGTAGGTGAACCTGCGG-3') and ITS4 (5' -TCCTCCGCTTATTGATATGC-3') (White et al. 1990). The DNA extract was amplified in a total reaction volume of 25 µL which contained; 10 µL (1x) Dream*Taq*™ DNA polymerase (Thermo Scientific™), 2 µL of template DNA (approximately 10-60 ng/µL), 1 µL of each primer (ITS1 and ITS4; Integrated DNA Technologies Inc., Australia) and 11 µL of ultra-pure water (Life Technologies, Thermo Fisher Scientific Inc., USA). A negative control was included where 2 µL of water was added instead of the DNA template. The PCR was performed using a DNA Thermal Cycler (BIO-RAD, CA, USA), and the conditions for all samples were: an initial denaturation step at 95°C for 3 min, followed by 35 cycles of 30 s at 95°C, 30 s at 54°C, and 1 min at 72°C, and a final extension at 72°C for 7 min. The resulting products were stored at -20^{o}C until used. Amplification success was determined by running 6 µL aliquots of the PCR in a 1% agarose gel utilizing 1xTris-Acetate-EDTA (TAE) buffer at 90V for 45 min. A 1 kb Plus DNA ladder™ (6 ng/µL; (Life Technologies, Thermo Scientific Fisher Scientific, Inc., USA) molecular weight marker was loaded in the first and last lanes, and the agarose gel was stained with GelRed® (Biotium, USA). Gels were viewed under UV light using the Versa Doc imaging system (Mode 3000, Bio-Rad, CA, USA), where a single bright band produced by each sample indicated successful amplification, with the 1 kb plus DNA ladder used to determine the expected base pair (bp). The expected size of the amplified product was approximately 450-600 bp. The final PCR products were sent for sequencing at the Lincoln University sequencing facility using the ABI Prism 3130x1 Gene Analyzer with a 16 capillary 50 cm array, using performance optimized polymer 7 (POP7).

2.2.4.3 Analysis of DNA sequences

The forward and reverse DNA sequences received were manually trimmed for inconsistencies based on the chromatogram using the BioEdit™ sequence alignment editor 7.2. The consensus sequences were then generated from the trimmed forward and reverse sequence and submitted to the GenBank database (https://blast.nlm.nih.gov/Blast.cgi) to determine identity using the basic local alignment search tool (BLASTN) (Version2.7.1). Species identity was selected based on sequence identity of 98% or above, and if the marching sequence had been deposited either by a reputable researcher active in this area or was described in a referred publication.

2.2.5 Statistical analysis

Data were analysed using GenStat statistical package (version 12 edition) (Payne et al. 2009). Two-way analysis of variance (ANOVA) in randomised block design was conducted to compare means between

isolates and treatments. The mean conidial length and width, colony diameter, and spore production were compared between isolates and treatments to determine significant difference at the 95% Confidence Interval. Fisher's protected least significant difference test and Bonferroni tests were used to compare significance between, isolates or and treatments that differed statistically from each other. Regression statistics at p=0.05 were run to establish the statistical relationship between mean colony growth and conidia production at different temperatures and light regimes. Data on colony colour, conidia shape, appressorial shape, and presence or absence of setae were presented using descriptive statistics in a tabular form. Bar charts and tables were used to show colony growth and spore production trends of isolates under the two light regimes and varying temperatures.

2.3 Results

Five *Colletotrichum* species isolates were used for the experiment Three isolates, C.g1, C.g2, and C.g3 were recovered from apple fruit, and two isolates, C.a4 and C.a5, from avocado fruit. Morphological characteristics and molecular sequencing were used to identify the *Colletotrichum* isolates to species level.

2.3.1 Morphological characteristics of *Colletotrichum* species isolates

Morphological characteristics including colony color, conidial shape, conidial length, and width, appressorial shape, presence or absence of perithecia and setae, colony growth rate, and sporulation capacity of the five isolates (C.g1, C.g2, C.g3, C.g4, and C.a5) were determined and are described in the following sections.

2.3.1.1 Colony color

The isolates exhibited a wide range of colors on the upper and the reverse side of the plates (Table 2.1). The colony color of isolate C.g1 on both the upper and reverse sides varied from sulfur yellow to amber (Figure 2.1 A, B). Isolate C.g2 produced colonies of greyish sepia in color on PDA both for the upper and reverse sides of the colony (Figure 2.1 B). Colonies of isolate C.g3 were pale mousey grey in color, and isolate C.a4, sky grey on the upper, and lavender grey color on the reverse side of the colonies. Isolate C.a5 exhibited distinct colony characteristics that varied from those of the other isolates. The colonies had a smoky grey color in the center of the culture with a rose color radiating from the center to the edge of the plate. The reverse side of the colony was pink in color.

2.3.1.2 Conidial shape

The shape of the conidia of isolates C.g1 and C.g3 were cylindrical with blunt ends (Table 2.1, Figure 2.1a). The conidia of isolate C.g2 were short with rounded ends. Conidia of isolates C.a4 and C.a5 were short in length, fusiform with both ends being sharp (Table 2.1, Figure 2.1).

2.3.1.3 Conidial size

All isolates produced conidia ranging from 8.1 to 15.1 µm in length and 3.1 to 5.4 µm in width (Table 2.1). The mean length and width of conidia of isolates C.g1 and C.g3 were greater (1.9 x 4.3 µm and 11.58 x 4.5 µm, respectively) than for the other isolates. Isolate C.g2 produced the shortest conidia (mean 9.8 µm) compared with other isolates (Table 2.1).

2.3.1.4 Appressorium development

Appressoria were produced by all isolates after 21 days of incubation on the coverslips (Table 2.1, Figure 2.1 E). The appressoria were sub-globose to clavate in shape, which was either lobed or not lobed. Appressoria of isolates C.g1, C.g2, and C.g3 were similar in shape and slightly more lobed/clavate than those produced by isolates C.a4 and C.a5.

2.3.1.5 Perithecial morphology

Four isolates (C.g2, C.g3, C.a4 and C.a5) produced perithecia (Table 2.1, Figure 2.1G). Isolate C.g1 did not produce perithecia on PDA (Table 2.1). Perithecia were formed in clumps and were rounded, or flask-shaped, pigmented, with well-developed ostioles. The shape of the perithecia of all four isolates that produced perithecia was similar.

2.3.1.6 Setae formation

All isolates apart from isolate C.g1 produce setae in culture (Table 2.1). The setae were melanized, multi-septate, and sparse to abundant in culture (Figure 2.1 G). Sparse setae was observed in the cultures of isolates C.g1, C.g3, and C.a4, while they were abundant for isolates C.g2 and C.a5. Setae of most isolates were cylindrical with rounded apices. Setae became hyaline at the tip during the incubation period and began to produce conidia.

Based on these morphological characteristics and comparison to species descriptions by (González *et al.*, 2006a; Hassan *et al.*, 2018; Munir *et al.*, 2016b), isolates C.g1, C.g2 and C.g3 were provisionally identified as *Colletotrichum gloeosporioides* species complex and isolates C.a4 and C.a5 provisionally identified *C. acutatum* species complex.

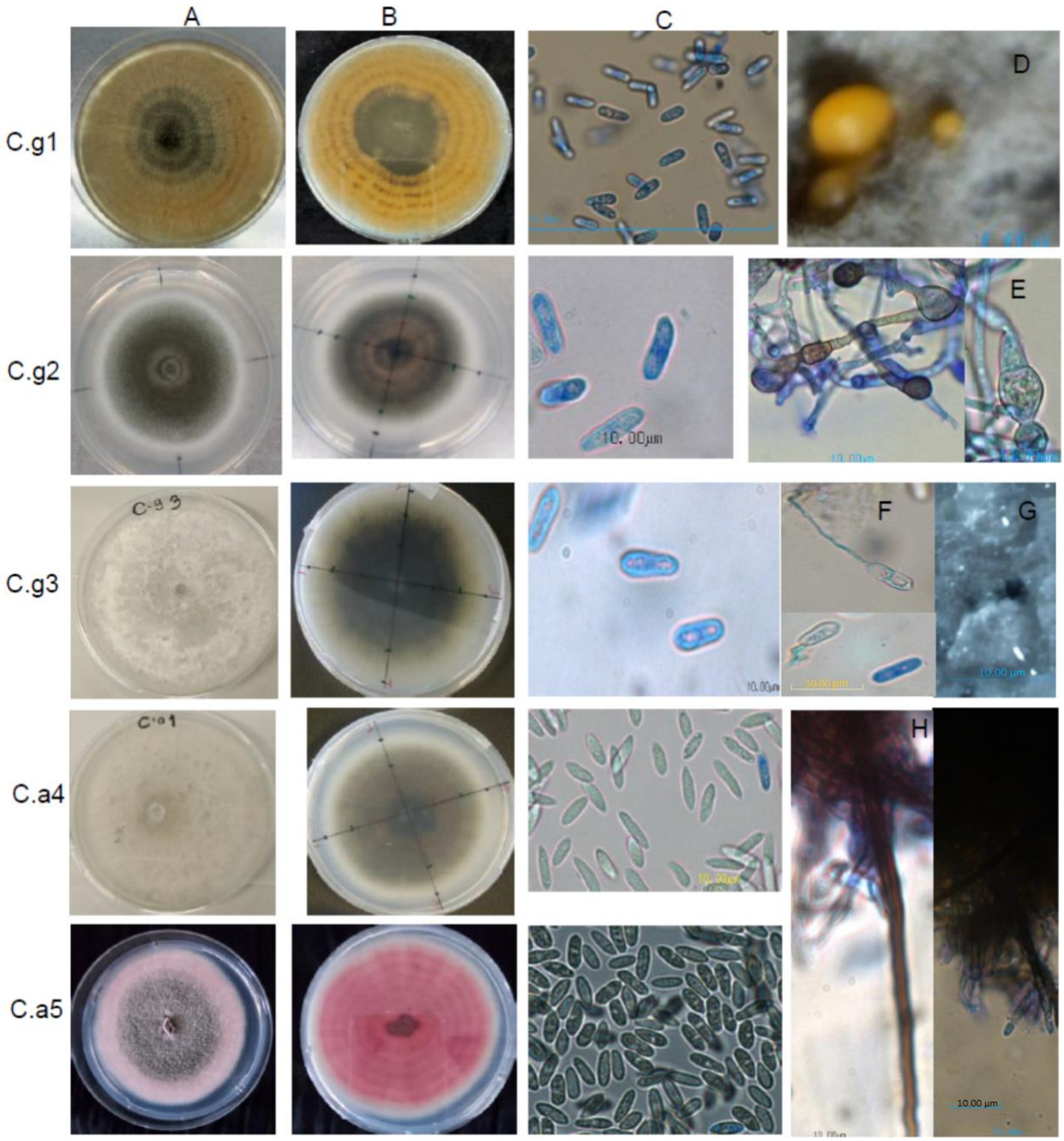

Figure 2. 1 Morphology and cultural characteristics of *Colletotrichum* species isolates recovered from avocado and apple fruit assessed after 192 hr and 336 hr. (A) Upper and (B) reverse view of the colony on PDA; (C) micrographs of conidia of *Colletotrichum* species isolates; (D) conidiomata; (E -F) micrographs of appressoria of *Colletotrichum* species; (G) micrograph of perithecia of *Colletotrichum* species isolate C.g2; (H) micrographs of setae of *Colletotrichum* species isolates. Scale bars = 10 µm for C; 20 µm for D-H.

Table 2. 1 Morphological characteristics of *Colletotrichum* species isolates determined after incubation at 25°C in 12: 12 hr photoperiod on potato dextrose agar.

Isolate	Conidial shape	Conidial size						Appresorial shape	Perithecia (+/-)	Setae (+/-)	Colony color
		Length (µm)			Width (µm)						
		Min	Max	Mean*	Min	Max	Mean*				
C.g1	Cylindrical with blunt ends	9.02	14.91	11.97 a	3.59	4.97	4.28 a	Lobed	-	-	Sulphur yellow, Amber
C.g2	Short and rounded ends	8.04	11.57	9.81 c	4.97	4.43	4.7 a	Globose, subglobose	+	+	Greyish sepia
C.g3	Cylindrical with one blunt end	8.10	15.05	11.58 a	3.87	5.43	4.65 a	Globose, subglobose	+	+	Pale mouse grey
C.a4	Short with both ends sharp	9.65	12.78	11.22 a	3.08	3.73	3.41 b	Subglobose, lobed	+	+	Sky grey, lavender grey
C.a5	Fusiform with sharp ends	10.14	11.78	10.96 b	4.01	4.27	4.14 a	Globose, subglobose	+	+	Rose, pinkish

Means followed by different letters are significantly different at the p=0.05 level.
Presence (+) or absence (-) of perithecia or setae on 14-day-old culture on PDA.

The results of the preliminary experiment to determine the best media for colony growth and spore production of isolates incubated at 20 and 25°C showed MEA to support the fastest growth and maximum spore production and thus was selected for the main experiment (Appendix A, Table A.3).

2.3.1.7 Growth rate of cultures

The mean colony diameter (mm) of isolates incubated at the different temperatures and under the two light regimes was recorded at 48 hr intervals between 48-336 hr. At 25°C and 30°C and light: dark conditions isolate C.g1, C.g2 and C.g3 had reached the edge of the plates by 192 hr to 288 hr. Therefore, comparisons of the effect of temperature and light regime on the growth of the different isolates were made at the 192 hr assessment time (full data shown in Appendix A, Table A.4). The colony diameter was significantly affected (p=0.001 Appendix A, Table A.5) by the interaction between the isolate and light regime. In all cases, the mean colony diameter of the isolates was significantly higher when incubated under light: dark conditions compared with under constant dark incubation (Figure 2.2). In light: dark incubation, the mean colony diameter differed significantly (p=<0.001; Appendix A, Table A.7) between all isolates, with the colony diameter of isolate C.g1 (45.11 mm) being significantly greater compared with all other isolates, and colony diameter of isolate C.a4 being significantly less (34.36 mm) compared with all other isolates (Figure 2.2, Appendix A, Table A.6). In contrast under constant dark incubation, the mean colony diameter of the isolates did not differ significantly (p=0.598; Appendix A,

Table A.8), with the colony diameter ranging from 3.33 mm for isolate C.g2 to 3.08 mm for isolate C.a4 (Figure 2.2; Appendix A, Table A.6).

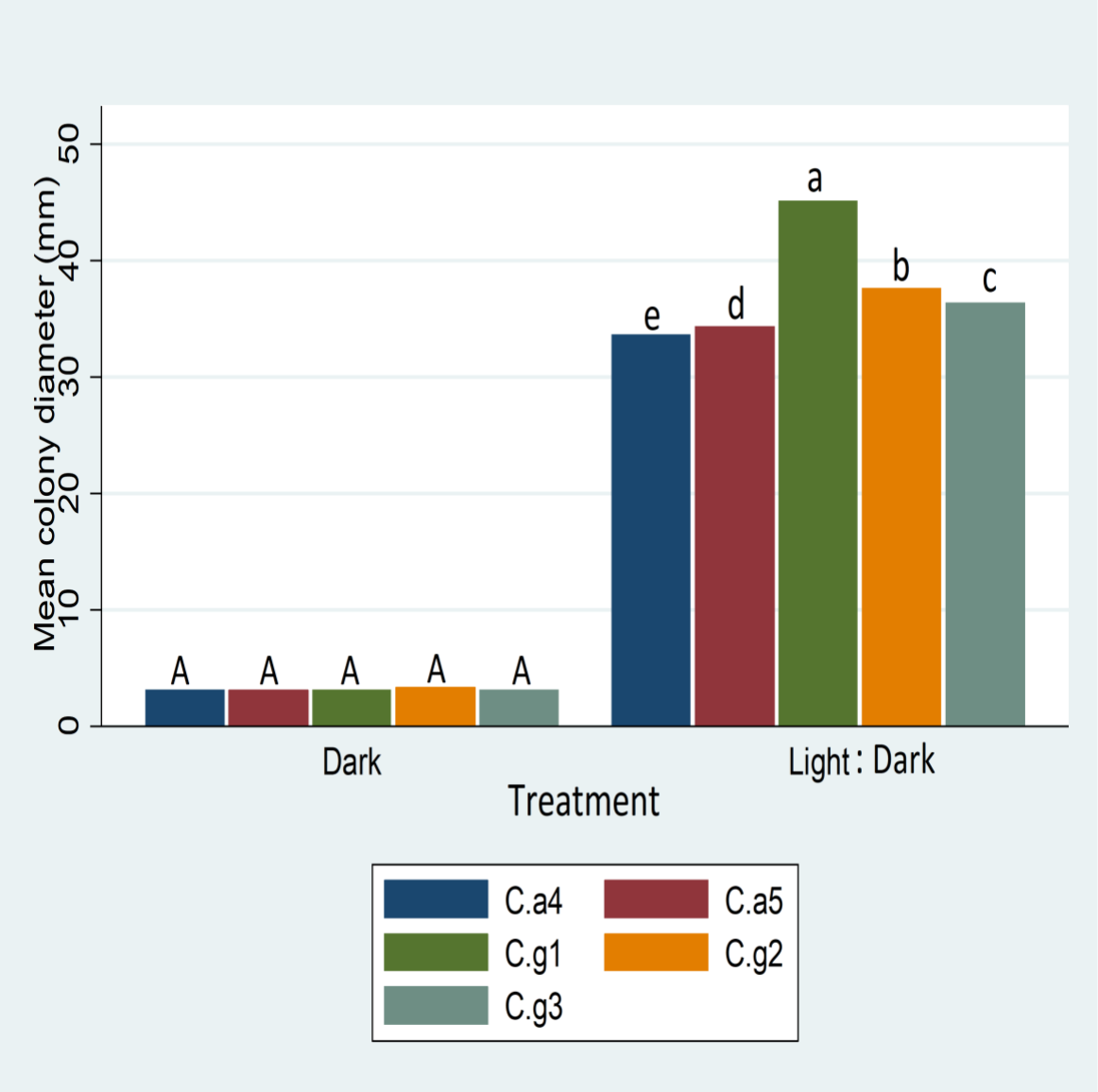

Figure 2. 2 Mean colony diameter (mm) of *Colletotrichum* species isolates incubated on malt extract agar under 12 hr light: 12 hr dark (Light: Dark) and constant dark assessed after 192 hr. Means for light: dark with the same lower case letters and constant dark with upper case letters are not significantly different at p=0.05.

Colony diameter was significantly affected by the interaction between temperatures and isolate for both light: dark (p=<0.001; Appendix A, Table A.7) and dark (p=<0.001; Appendix A, Table A.8) incubation conditions. For incubation under light: dark conditions, the smallest colony diameter for all isolates was seen at 15°C and the largest colony diameter at 25°C for isolates C.g1, C.g2, and C.g3 and at 30°C for

isolates C.a4 and C.a5 (Figure 2.3 A). There was a significant effect (p=<0.001; Appendix A, Table A.7) of temperature on overall colony diameter, with colony diameter being significantly larger at 25°C (61.77 mm) than at all other temperatures, followed by 30°C (42.39 mm) which was significantly larger than at 20°C (30.33 mm) and the smallest at 15°C (15.19 mm) (Appendix A, Table A.9).

Under constant-dark incubation, the smallest colony diameter for all isolates was seen at 15°C and the largest colony diameter at 25°C for isolates C.g1 and C.g2, at 20°C and 25°C for isolates C.g3 and C.a5 and at 20°C, 25°C, and 30°C for isolate C.a4 (Figure 2.3 B). The diameters of C.a4 are not significantly different at 25°C and 30°C but significantly less at 20°C. There was a significant main effect of temperature (p=0.001: Appendix A, Table A.8) on the mean colony diameter of isolates with the colony diameter at 25°C (4.65 mm) being significantly larger than at all other incubation temperatures, followed by 20°C (3.43 mm) which was significantly larger than at 30°C (2.54 mm) and the smallest at 15°C (1.95 mm) (Appendix A, Table A.10).

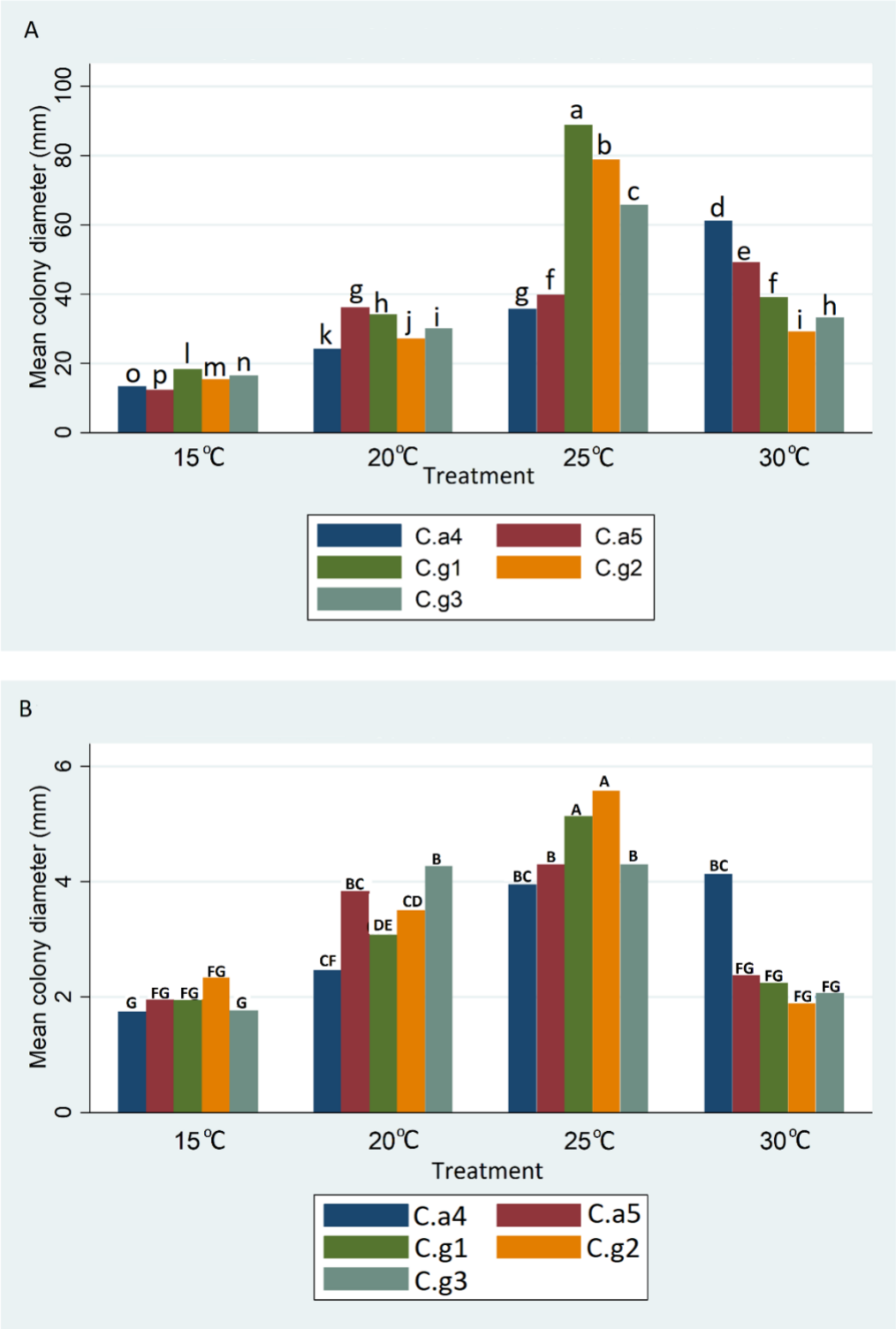

Figure 2. 3 Mean colony diameter (mm) of different *Colletotrichum* species isolates incubated in (A) 12 hr light: 12 hr dark and (B) constant-dark at varying temperatures on malt extract agar assessed after 192 hr. Means with the same letters are not significantly different at p=0.05.

2.3.1.7 Spore production

Since most isolates reached maximum colony diameter at 25°C and 30°C between 192 hr and 366 hr, comparisons were made at 192 hr for the effect of temperature on spore production of the different isolates (full data shown in Appendix A, Table A.11). There was a significant interaction (p=0.001, Appendix A, Table A.12) between the isolate and light regime on the mean spore production. The mean spore production of isolates was significantly higher when incubated under light: dark conditions compared with under constant dark incubation (Figure 2.4). In light: dark incubation, there was a significant difference (p=0.001; Appendix A, Table A.14) between isolates in mean spore production, with the mean spore production of isolate C.g1 being significantly higher (146.5 x 10^4 spore/mL) than all other isolates, and spore production of isolate C.g2 being significantly less (19.1 x 10^4 spore/mL) than all other isolates (Figure 2.4, Appendix A, Table A.13). Similarly, under constant dark incubation, there was a significant difference in the mean spore production (p=0.001; Appendix A, Table A.15) between isolates with the mean spore production of isolate C.a4 being significantly greater (22.8 x 10^4 spore/mL) than all other isolates. Of those isolates, isolate C.a5 produced a significantly greater (10.2 x 10^4 spore/mL) number of spores than all of the others (Figure 2.4; Appendix A, Table A.13).

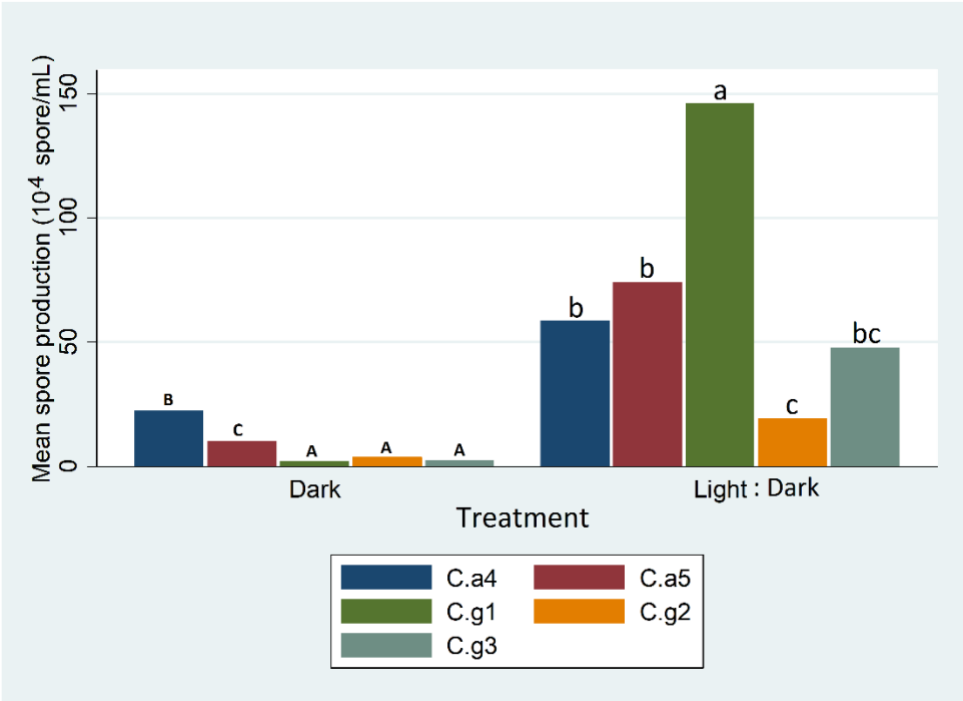

Figure 2. 4 Mean spore production (10^4 spore/mL) of *Colletotrichum* species isolates incubated on malt extract agar under 12 hr light: 12 hr dark (Light: Dark) and constant dark assessed after 192 hr. Means for light: dark with the same lower case letters and constant dark with upper case letters are not significantly different at p=0.05.

Spore production was significantly affected by the interaction between temperatures and isolate in light: dark (p=< 0.001; Appendix A, Tables A.14) and constant dark (p=< 0.001; Appendix A, Tables A.15) incubation. The mean spore production of isolates was significantly higher when incubated under light: dark conditions than under constant dark. In light conditions isolate C.g1 produced the greatest number of spores (146.5 x10^4 spore/mL) followed by isolates C.a5, C.a4, C.g3, with isolate C.g2 producing the lowest number of spores (Appendix A, Table A.13). Under dark incubation, spore production differed significantly between isolates, with C.a5 producing the highest number of spores (22.9 x10^4 spore/mL) compared with the other isolates (Table 2.2).

Table 2. 2 Effect of incubating different *Colletotrichum* species isolates under 12 hr light: 12 hr dark (Light: Dark) compared with constant dark on the mean spore production (10^4 spore/mL) on malt extract agar assessed after 192 hr.

Isolate	**Mean spore production (10^4 spore/mL)**	
	Light: Dark	Dark
C.g1	146.5 a	2.0 a
C.g2	19.1 c	3.6 a
C.g3	47.9 bc	2.3 a
C.a4	58.6 b	22.9 b
C.a5	74.1 b	10.2 c

*Means in each column followed by the same letter are not significantly different at p=0.05.

There was significant effect of temperature on the mean spore count of isolate in both light: dark (p= < 0.001; Appendix A, Table A.14) and constant dark incubation (p= < 0.001; Appendix A, Table A.15). In the light: dark regime, there was significantly higher spore production (146.06 x 10^4 spore/mL) at 25^{o}C than at all other temperatures. Spore production at 20^{o}C (49.20 x 10^4 spore/mL) and 30^{o}C (69.22 x 10^4 spore/mL) did not differ significantly, with both being higher than 15^{o}C (12.54 x 10^4 spore/mL) (Appendix A.16). Similarly, under continuous dark incubation conditions there was significantly higher spore production (17.1 x 10^4 spore/mL) at 25^{o}C than at all other temperatures. Spore production at 20^{o}C (7.75 x 10^4 spore/mL) and 30^{o}C (7.01 x 10^4 spore/mL) did not differ significantly, with both being higher than 15^{o}C (0.93 x 10^4 spore/mL) (Appendix A.17).

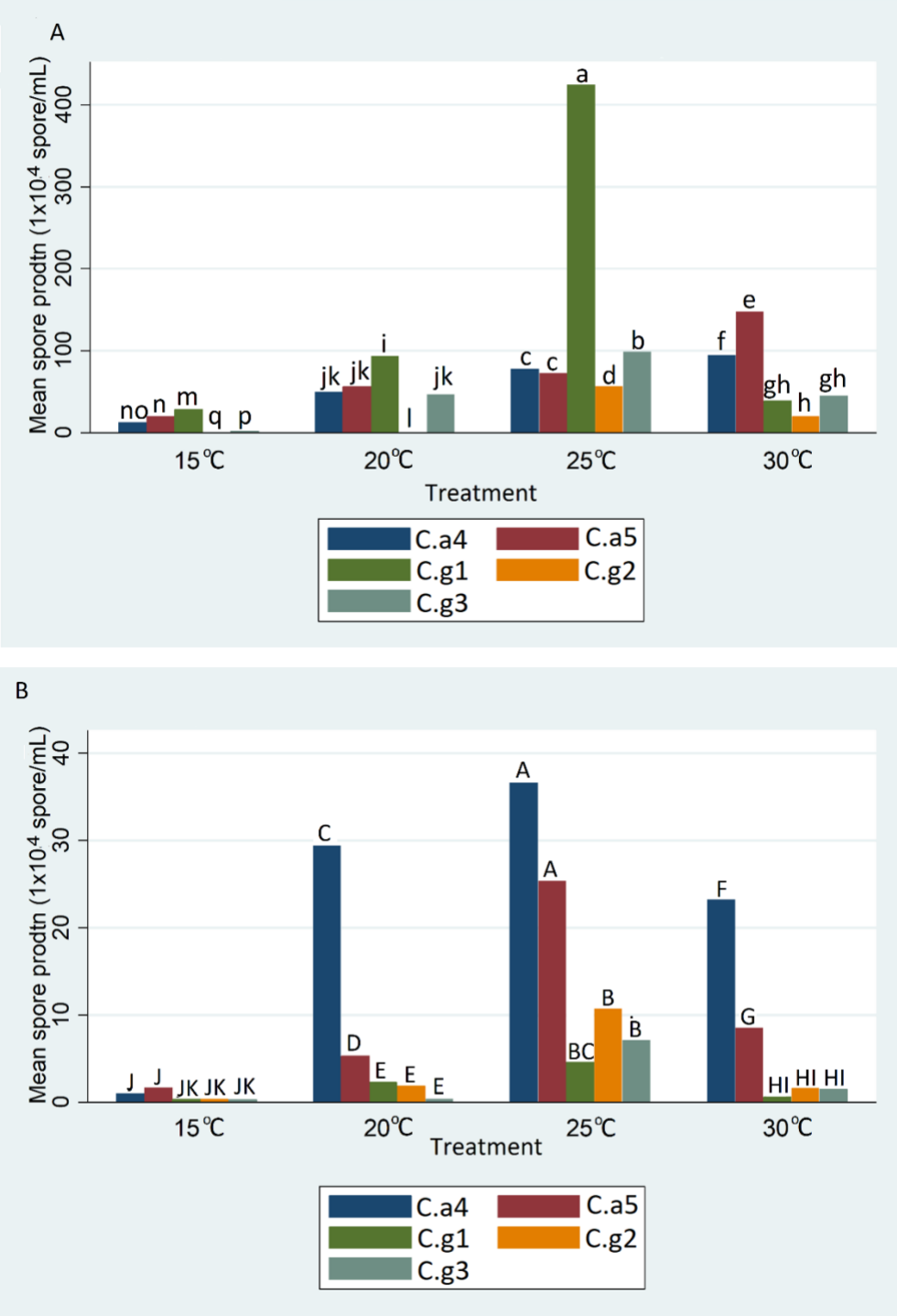

Figure 2. 5 Mean spore production (x 10^4 spore/mL) of different *Colletotrichum* species isolates incubated in (A) 12 hr light: 12 hr dark, and (B) constant dark at varying temperatures on malt extract agar assessed after 192 hr. Means for light: dark with the same lower case letters and constant dark with upper case letters are not significantly different at p=0.05.

2.3.2 Relationship between spore production and colony diameter of *Colletotrichum* species isolates under light:dark conditions

The relationship between spore production and mycelial growth of the five isolates was determined after 192 hr at 25°C because this temperature supported the largest colony diameter and highest spore production for most isolates. For isolate C.g1 there was a significant correlation (r=0.68; p=0.03; Appendix A, Table A.18 a) between spore production and colony diameter (Figure 2.2. A). For all other isolates (C.g2, C.g3, Ca4, and C.a5), there was no significant relationship (p=>0.05, Appendix A, Table A.18 b, c, d, and e) between spore production and mycelial growth (Figure 2.6 B, C, D, and E). However, analysis at 336 hr, showed all isolates to have a significant correlation (p=<0.05, Appendix A, Table A.19) between spore production and colony diameter (Figure 2.7 A, B, C, D, and E)

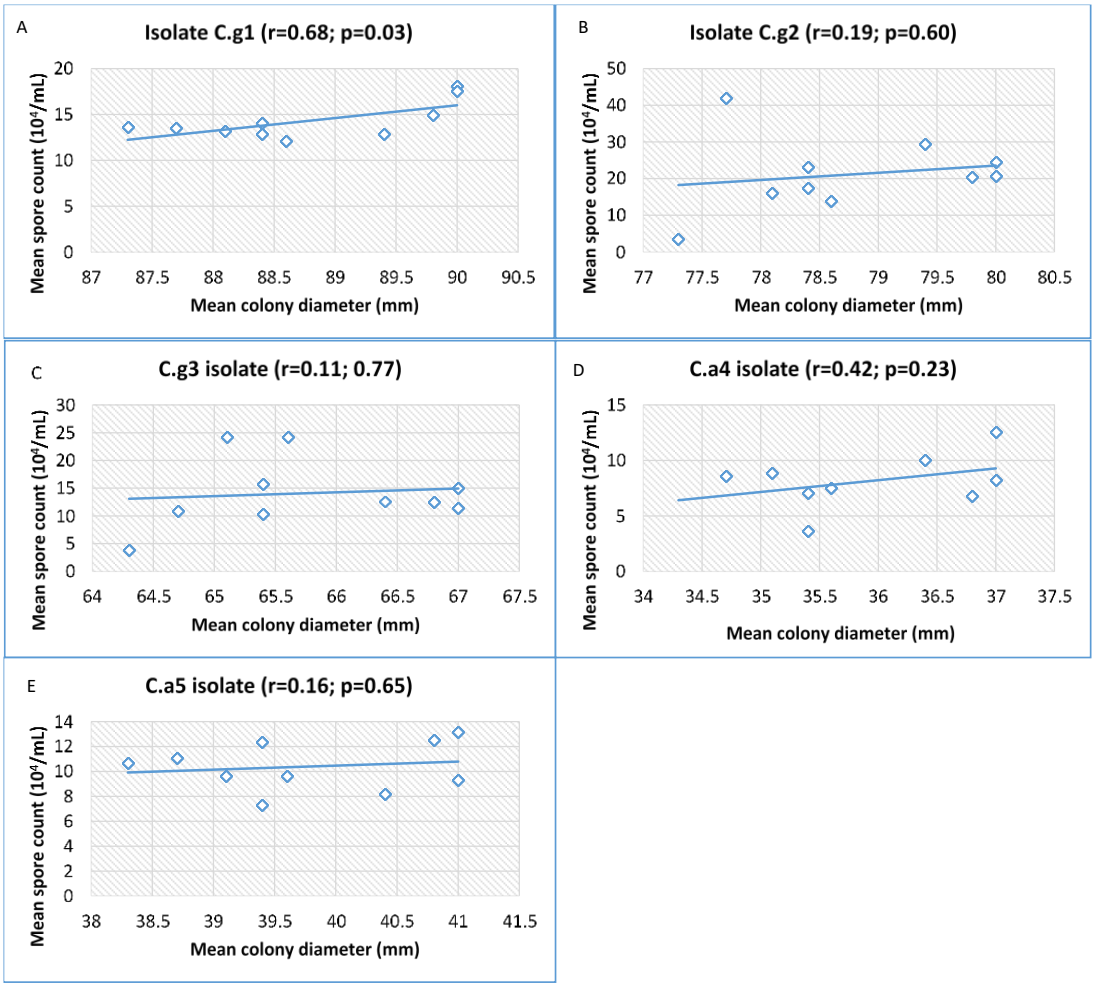

Figure 2. 6 Scatterplot showing the relationship between colony diameter (mm) and spore production (x 10^4 conidia/mL) for five *Colletotrichum* species isolates incubated under 12hr:12 hr light: dark regime at 25°C and assessed after 192 hr.

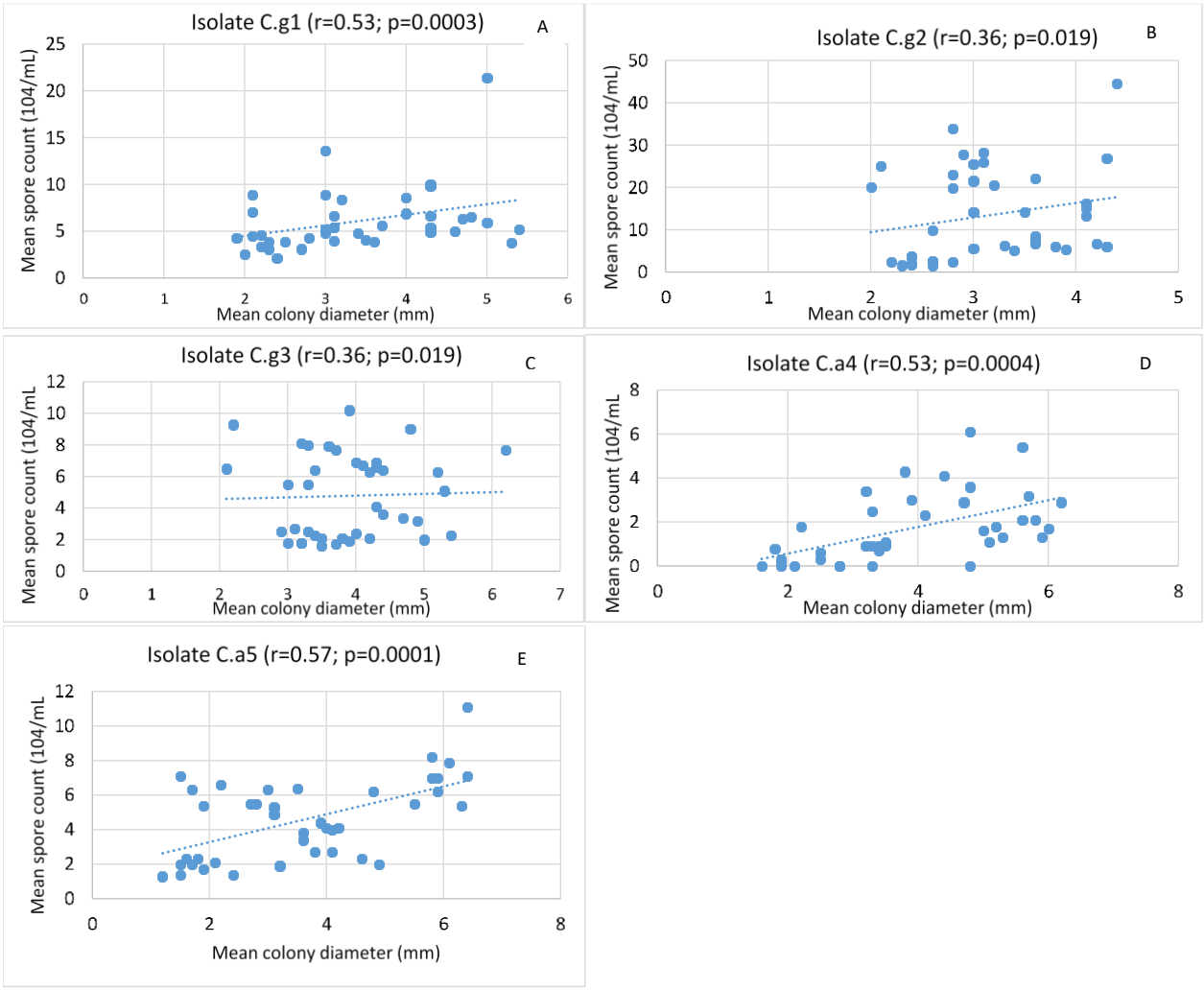

Figure 2. 7 Scatterplot showing the relationship between colony diameter (mm) and spore production (x 10^4 conidia/mL) for five *Colletotrichum* species isolates incubated under 12hr:12 hr light:dark regime at 25°C and assessed after 336 hr.

2.3.3 Relationship between spore production and colony growth of *Colletotrichum* species isolates under continuous dark.

There was no significant correlation between spore production and colony diameter under continuous dark incubation at 25°C and assessed after 192 hr for any of the five *Colletotrichum* species isolates (p=>0.05; Appendix A, Table A.20 a, b, c, d, and e) (Figures 2.8 A, B, C, D, and E).

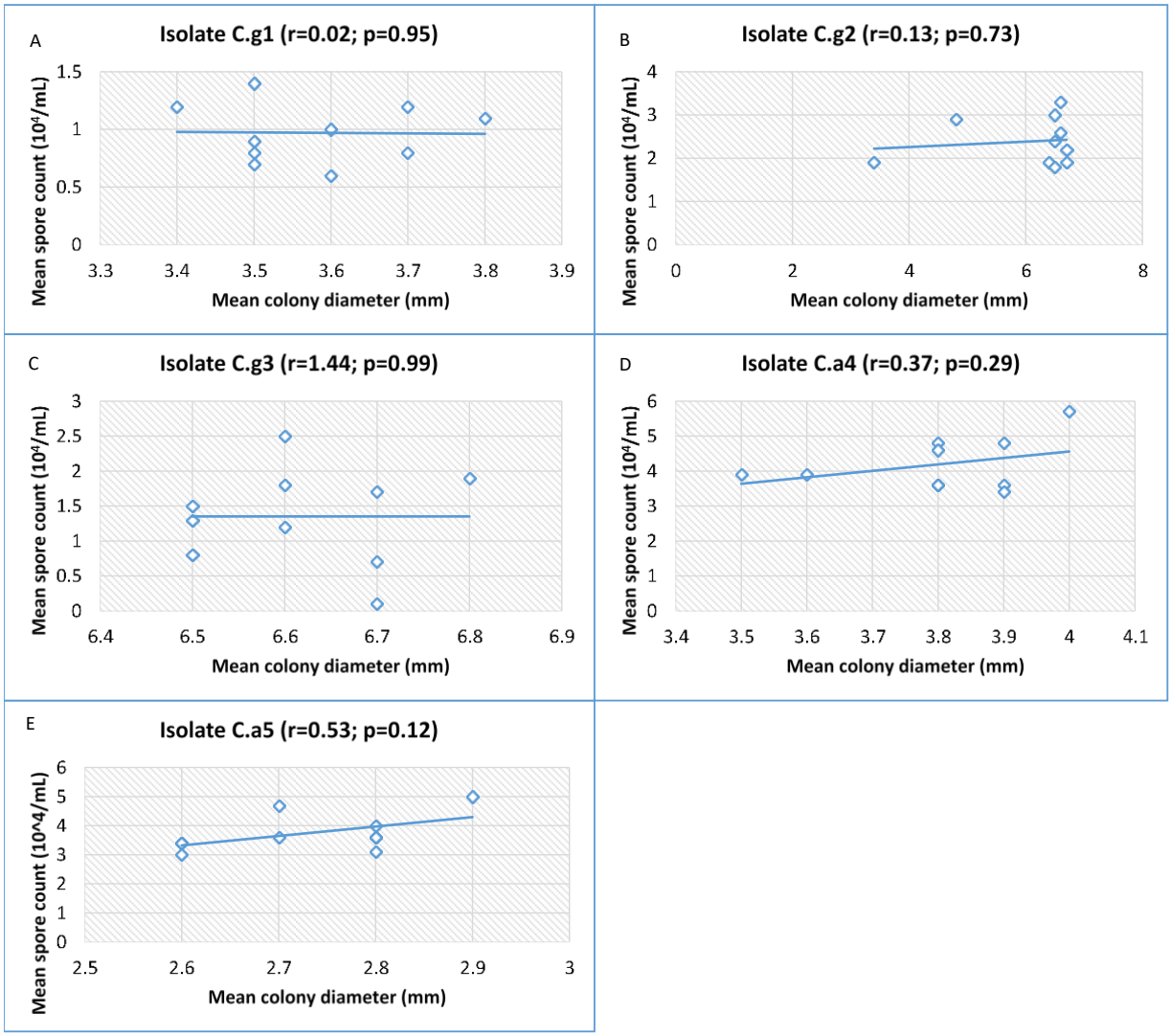

Figure 2. 8 Scatterplot showing the relationship between colony diameter (mm) and spore production (x 10^4 conidia/mL) for five *Colletotrichum* species isolates incubated under continuous dark at 25°C and assessed after 192 hr.

2.3.4. Molecular identification of isolates recovered from infected fruit

For all the isolates, DNA amplification with the universal ITS primer pairs ITS1/ITS4 produced a band of approximately 500 – 600 bp (Figure 2.9). Sequences of isolates C.a4 and C.a5, morphologically identified as *C. acutatum* species complex, showed 100% sequence identity with the sequences of *Colletotrichum acutatum* and *Colletotrichum fioriniae* obtained from the NCBI database (Table 2.2; Appendix A.21). Since *Colletotrichum fioriniae* is part of the *C. acutatum* species complex, with the ITS gene region not being sufficient to resolve these two species, these isolates were identified as *C. acutatum* species complex Barquero Quirós *et al.* (2013); (Ciampi-Guillardi *et al.*, 2014; De Silva *et al.*, 2017a). Sequences of isolates C.g1, C.g2, and C.g3, morphologically identified as *C. gloeosporioides* species complex, showed 100% sequence identity with the sequences of *C. gloeosporioides* and *Colletotrichum kahawae* obtained from the NCBI database (Table 2.2; Appendix A.21). Similarly, since *Colletotrichum kahawae* is part of the *C. gloeosporioides* species complex, with the ITS gene region not being sufficient to resolve these two species, these isolates were identified as *C. gloeosporioides* species complex (Weir *et al.*, 2012c).

Table 2. 3 BLAST results for the sequences of the ITS gene region of isolates morphologically identified as *Colletotrichum acutatum* (C.a4 and C.a5) and *Colletotrichum gloeosporioides* (C.g1, C.g2, and C.g3).

Isolate	Source	Identity	Query cover	E-value	% Identity	GenBank Accession No	Length (bp)
C.g1	Apple	*Colletotrichum gloeosporioides*	100%	0	100	MF495411.1	599
		Colletotrichum kahawae	100%	0	100%	MN744289.1	
C.g2	Apple	*Colletotrichum gloeosporioides*	100%	0	100	MT083978.1	593
		Colletotrichum kahawae	100%	0	100	MN744289.1	
C.g3	Apple	*Colletotrichum gloeosporioides*	100%	0	100	MF495411.1	599
		Colletotrichum kahawae	100%	0	100	MT133276.1	
C.a4	Avocado	*Colletotrichum acutatum*	100%	0	100	AB042300.1	584
		Colletotrichum fioriniae	100%	0	100	MN588150.1	
C.a5	Avocado	*Colletotrichum fioriniae*	100%	0	100	MT133290.1	596
		Colletotrichum acutatum	100%	0	100	MN586423.1	

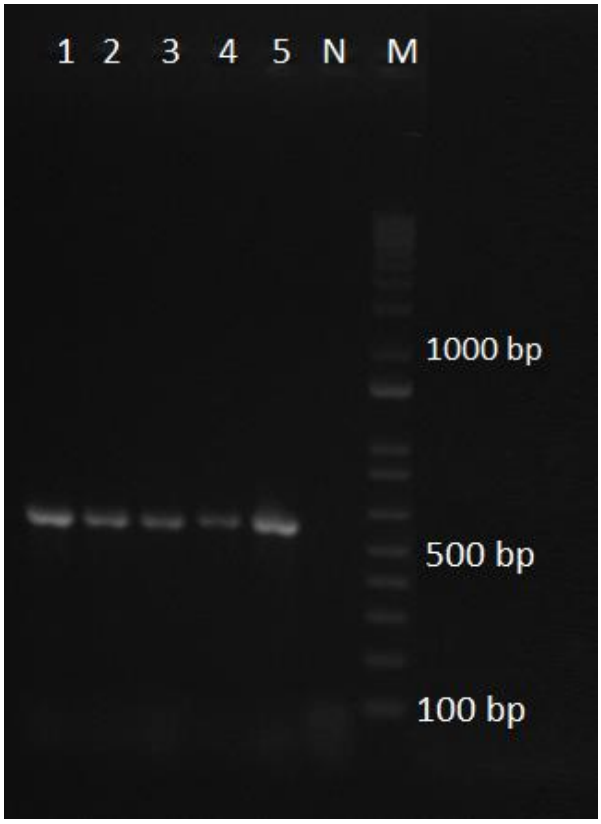

Figure 2. 9 PCR products of the amplified DNA from different *Colletotrichum* species isolates using the Universal primer pairs ITS1/ITS4. Lanes 1 - 5 PCR products of isolates C.g3, C.g2, C.g1, C.a5, and C.a4; N is a negative control (water); M molecular weight marker (1 kb + ladder).

2.4 Discussion

In this study, the morphological characteristics of five *Colletotrichum* species isolates recovered from naturally infected apple and avocado fruits were used to provide preliminary identifications of the isolates, with the identity confirmed by sequencing of the ITS gene region. Each species was recovered from a single host, with all three isolates recovered from apple fruit (C.g1, C.g2, and C.g3) identified as belonging to the *Colletotrichum gloeosporioides* species complex, while both isolates recovered from avocado fruit (C.a4 and C.a5) were identified as belonging to the *Colletotrichum acutatum* species complex. *Colletotrichum gloeosporioides* and *C. acutatum* species complexes have been reported earlier as causal agents for anthracnose in avocado and apple fruits both outside and within New Zealand (Hofer *et al.*, 2021; Munir *et al.*, 2016a; Nelson, 2008a; Sharma *et al.*, 2017; Silva-Rojas & Ávila-Quezada, 2011)

To identify the isolates obtained from the infected fruits, cultural features such as colony colour, conidia shape and size, setae formation, appressorial development, and perithecia morphology were used. The results showed that despite the limitations surrounding the application of colony and morphological characteristics in identifying *Colletotrichum* species, these remain a useful basis for fungal species identification, especially where sequencing methods are unavailable. When used appropriately, specific cultural characteristics such as colony colour, growth rate, and conidia size and shape can provide a preliminary genus or species identification, and a means for grouping isolates based on key features for subsequent species identification using other techniques such as sequencing taxonomic informative gene regions as used in the present study. However, certain characteristics such as the production of setae or perithecia, or the shape of appressoria were shown in the current and other studies (Crouch, 2009) to not be informative to separate *C. acutatum* and *C. gloeosporioides*. Therefore, for preliminary species identification, future research should make use of informative features such as colony colour, conidia shape and size, and growth rate. The morphological features of *C. gloeosporioides* and *C. acutatum* isolates in the present study were similar to those previously described by Hassan *et al.* (2018); (Xie *et al.*, 2010; Zhang *et al.*, 2021), and Damm *et al.* (2012).

The morphological identifications were confirmed by molecular sequencing. Sequencing of the ITS gene region showed that isolates morphologically identified as *Colletotrichum acutatum,* isolates C.a4 and C.a5, had 100% sequence identity with sequences of *Colletotrichum acutatum* and *Colletotrichum fioriniae* obtained from the NCBI database. *Colletotrichum fioriniae* is a member of the *C. acutatum* species complex and therefore, these isolates were identified as *C. acutatum* species complex since the

ITS gene region is reported to not be able to sufficiently resolve these two species (Barquero Quirós *et al.*, 2013; Ciampi-Guillardi *et al.*, 2014; De Silva *et al.*, 2017a). Similarly, isolates morphologically identified as *C. gloeosporioides,* isolates C.g1, C.g2, and C.g3 had 100% sequence identity with the sequences of *C. gloeosporioides* and *Colletotrichum kahawae* obtained from the NCBI database. Similarly, since *C. kahawae* is part of the *C. gloeosporioides* species complex, with the ITS gene region unable to sufficiently resolve these two species, these three isolates were identified as *C. gloeosporioides* species complex (Weir *et al.*, 2012c). Sequencing of additional gene regions such as partial Actin (ACT), β-tubulin (TUB2), Calmodulin (CAL), Glutamine synthetase (GS), and Glyceraldehyde 3-phosphate dehydrogenase (GPDH) was stated by Cai *et al.* (2009) to be required to discriminate between closely related species, especially within these species complexes (Doyle *et al.*, 2013b). This present study was limited to characterising the isolates using only the universal primers due to time limitations. To elucidate further which of the species within these two species complex these isolates belong to, sequencing of the additional gene regions would be required.

From the results of the study, all *Colletotrichum* species isolates grew faster and produced more spores when incubated under 12 hr light: 12 hr dark conditions compared with in constant dark incubation. This is consistent with the observations of Khanzada *et al.* (2018), who reported a higher colony growth rate of *C. gloeosporioides* under 12 hr light: 12 hr dark incubation conditions compared with constant dark conditions. Similarly, Soltani *et al.* (2014) reported the growth and sporulation of *C. gloeosporioides* isolates to be highly dependent on light. In their study, at each of the tested temperatures, sporulation and colony growth of *C. gloeosporioides* was greater under 16/8 hr light/dark interval compared with under continuous dark. Further, the study of Kommula *et al.* (2017) revealed that the exposure of *Colletotrichum capsicii* isolates to alternate cycles of 12 hr light: 12 hr darkness resulted in greater mycelia growth compared to 24 hr exposure to either continuous light or dark. Although there has been a number of studies on *C. gloeosporioides,* this is the first study to investigate the effect of different light regimes on the growth and sporulation of *C. acutatum*. More than 100 fungal species, representing all phyla, have been found to be reactive to light (Tisch & Schmoll, 2010). Light regulates many metabolic activities, including circadian rhythms, asexual conidiation, pigmentation, secondary metabolism, and sexual development of fungi (Purschwitz *et al.*, 2006). However, most fungal species are reported to vary in their response to different light regimes (Idnurm *et al.*, 2010) and further research could be carried out to investigate the effect of different light regimes including light intensity on growth and sporulation of *Colletotrichum acutatum* and *C. gloeosporioides* isolates. This would provide information

on conditions that promote sporulation of these important plant pathogens that may aid in the development of effective control strategies of the pathogens in the field.

Colletotrichum gloeosporioides isolates (C.g1, C.g2, and C.g3) had greater colony growth compared with *C. acutatum* isolates (C.a4 and C.a5). This result is comparable to a report by Varzea *et al.* (2002) where the mycelial growth rate of *C. acutatum* was 5.7 mm/day at 30°C compared with 12.3 mm/day at 30°C for *C. gloeosporioides*. Similarly, Harp *et al.* (2008) reported isolates identified by species-specific PCR as *C. gloeosporioides* to grow faster (5.91 and 5.93 mm/day) than those identified as *C. acutatum* (2.96 and 3.54 mm/day). This difference in growth rates of *C. acutatum* and *C. gloeosporioides* under very specific defined incubation conditions could be used to tentatively separate these two species. Other researchers Brown *et al.* (1996); (Hadden, 1989; Kim *et al.*, 1986; Marvel *et al.*, 2003; Sutton, 1992) have also suggested that the relative colony growth rate of isolates can be of taxonomic significance.

In the present study, temperature was shown to affect colony growth and sporulation of isolates of both *C. acutatum* and *C. gloeosporioides* under both light:dark and dark incubation conditions. Within the temperature range tested, minimum colony growth and spore production for all isolates was observed at 15^{o}C with maximum growth and spore production observed at 25^{o}C for *C. gloeosporioides* isolates (C.g1, C.g2, and C.g3) and 30^{o}C for *C. acutatum* isolates (C.a4 and C.a5) under 12 hr light: 12 hr dark incubation. A similar effect of temperature was seen on colony growth and spore production when the isolates were incubated under constant-dark conditions although most isolates grew similarly at 20-30^{o}C. This finding is comparable with the results of Sangeetha and Rawal (2010) and Kumara and Rawal (2010) where the growth of *C. gloeosporioides* isolates was slowest at 15^{o}C, with 25-28°C being optimum for growth and sporulation. Similarly, Morkeliūnė *et al.* (2021) reported the colony growth of *C. acutatum* to be slow at temperatures below 15^{o}C, while for *C. gloeosporioides* Khanzada *et al.* (2018) recorded limited growth at incubation temperatures of 15°C, 40°C, and 45°C. These reports demonstrate the inability of these pathogen species to grow at both low and high temperatures. In contrast to the results of the current study which indicated that the optimum growth temperature for *C. acutatum* was 30^{o}C and *C. gloeosporioides* was 25^{o}C, Kenny *et al.* (2012) reported that the optimum temperature for an isolate of *C. acutatum* was 21°C and an isolate of *C. gloeosporioides* was 29-31^{o}C. The difference in the temperature optima for *C. acutatum* and *C. gloeosporioides* isolates determined in the different studies could be due to the isolates used in the different studies representing different species within the respective species complexes that infect various hosts in different geographic regions. In the study by Kenny *et al.* (2012) the isolates of each species were recovered from symptomatic coffee berries in

Papua New Guinea, whilst in the current study, *C. acutatum* and *C. gloeosporioides* isolates were originally recovered from symptomatic apple and avocado fruit respectively, and thus may represent different species within these species complexes.

Only one isolate of *C. gloeosporioides,* isolate C.g1, showed a positive relationship between spore production and mycelia growth under 12 hr light: 12 hr dark incubation conditions at 25^{o}C after 192 hr with the larger colonies resulting in higher spore production. Soltani *et al.* (2014) reported a similar result where mycelial growth and sporulation of *C. gloeosporioides* was highly dependent on the light regime. However, for all the other isolates, it was unclear why this was not the case, but might be related to the maturity of the mycelium not being able to support conidial production at the 192 hr assessment period in contrast to C.g1 which produced the largest colonies at 25^{o}C and was also seen to produce the most spores. This suggestion is supported by the results at 336 hr, where a positive correlation between mycelial growth and sporulation was observed for all isolates. When incubated in constant darkness, there was no correlation between spore production and mycelial growth for any of the isolates, indicating that an increase in colony growth did not affect spore production. This is likely to be due to spore production for these isolates being reliant on light levels (Mello *et al.*, 2004; Soltani *et al.*, 2014).

2.4.1 Conclusion

Based on colony morphological characteristics and sequencing of the ITS gene region the isolates obtained from avocado were identified as *C. acutatum* species complex and those recovered from apple as *C. gloeosporioides* species complex. Since these pathogens are known to infect mango (Pardo-De la Hoz *et al.*, 2016), their pathogenicity on the green bean, *Phaseolus vulgaris* was determined in the next chapter to assess the potential of the bean plant to act as an alternative inoculum host for mango. Results from the present study also showed 25-30^{o}C as the optimum temperature for the production of spores for all the *Colletotrichum* species isolates assessed. This information was used to inform the incubation conditions to ensure sufficient spore production of the selected isolates for inoculating the bean plants in the subsequent chapters. From the findings of this study, the identification of *Colletotrichum* species using colony and culture morphologies is useful to initially classify isolates of this pathogen group, however, the use of a combination of different techniques, including sequencing of taxonomic gene regions, is required to fully delineate *Colletotrichum* species. Sequences of the ITS gene region, however, were shown to be unable to delineate between the closely related species within these *Colletotrichum* species complexes, as demonstrated in the present study. Sequencing of additional gene

regions such as partial Actin (ACT), β-tubulin (TUB2), Calmodulin (CAL), Glutamine synthetase (GS), and Glyceraldehyde 3-phosphate dehydrogenase (GPDH) is required to separate these closely related species.

Chapter 3. Pathogenicity of *Colletotrichum* species isolates on *Phaseolus vulgaris* under glasshouse conditions.

3.1 Introduction

Common bean (*Phaseolus vulgaris*) is an herbaceous annual crop which is grown extensively throughout tropical, subtropical, and temperate regions of the world. The largest producer of common bean in the world is Latin America followed by Africa (CGIAR, 2015). In Uganda, bean is grown on any available arable land. Bean matures early making it the most common annual crop planted with perennial crops such as mango especially at the early stage of orchard establishment to make the most use of available land. There are many bean varieties grown in Uganda. Recent breeding techniques have enabled the production of even more highly nutritive, early maturing, and drought-resistant cultivars such as 'Narobean', 'ROBA', 'K131', 'K132', and 'Nabe', which are commonly cultivated in a pure or mixed stand with other crops (PABRA, 2016).

Common bean is affected by a wide diversity of biotic and abiotic stresses during its cultivation and storage (Mourice & Tryphone, 2012; Schwartz & Pastor-Corrales, 1994) resulting in large yield and post-harvest losses. Among the biotic factors, bean anthracnose disease caused by *Colletotrichum* species are known to produce substantial production losses. Of the various species of *Colletotrichum* that infect bean plants, *Colletotrichum lindemuthianum* is reported as the most important disease of common bean under cool and humid environments where it may cause up to 100% yield loss (Padder *et al.*, 2017). The ability of other *Colletotrichum* species such as *C. gloeosporioides* and *C. acutatum* which are responsible for mango anthracnose (León *et al.*, 2018; Qin *et al.*, 2019) to infect common bean is not well understood with only limited investigations on their pathogenicity to bean (Chakraborty *et al.*, 2019a).

Since many *Colletotrichum* species can survive on a broad host range and can develop unique relationships with particular hosts to cause asymptomatic infections (Everett *et al.*, 2018), they may serve as inoculum sources for infection of other plants (Phoulivong *et al.*, 2012; Udayanga *et al.*, 2013). Because, in practice, common bean is commonly alternated with other crops in mango cropping systems in Uganda, its potential to act as a reservoir of inoculum for *Colletotrichum* species that causes anthracnose disease in mango is likely. This could explain the high prevalence of mango anthracnose disease in Uganda as infected bean plants could not only act as a primary source of inoculum but the

pathogens are also reported to survive on host plant debris for more than 10 months (Norman & Strandberg, 1997). Infection of mango plants at the early stage of the orchard establishment following the dispersal of conidia from alternate host plants could persist and affect the crop in the subsequent season, especially during flowering and fruit development.

Effective control of anthracnose disease presents an important step to increase mango production and to reduce post-harvest disease and subsequent economic losses in Uganda. Understanding the potential role of bean plants in driving the epidemiology of mango anthracnose disease during early orchard establishment requires investigation. Thus, the overall aim of the research in this chapter was to determine the pathogenicity of isolates of two *Colletotrichum* species known to affect mango, on common bean in a glasshouse experiment. *Colletotrichum acutatum* species complex isolates C.a4 and *C. gloeosporioides* species complex isolate C.g1 and C.g3, recovered from avocado and apple fruit in the previous chapter were selected for the glasshouse experiment. Different spore concentrations of the different *Colletotrichum* isolates were also tested.

3.2 Materials and methods

3.2.1 Media preparation and planting of beans.

Phaseolus vulgaris ´Blue Lake runner' seeds were obtained from King Seed Company Ltd (New Zealand) and planted into 1.5 L plastic pots containing approximately 450 g of potting mix (Appendix B, Table B.1). The seeds were sown in the potting mix at 5 cm depth in the center of each pot, 2 seeds per pot. A total of 120 pots were set up. For each pot, 300 mL of water was added to moisten the potting mix, immediately after sowing the seeds, and the moisture was subsequently maintained at field capacity (60%) based on readings from a Decagon soil moisture sensor. Pots were then placed on metal wire benches in a glasshouse at the Lincoln University nursery facility (Figure 3.1). All plants were pruned by trimming their tendrils using secateurs weekly to encourage bushing and to keep them at the 3-node stage. This also helped to prevent cross infection between the different treatments.

Figure 3. 1 *Phaseolus vulgaris* seedlings growing in the glasshouse on wire benches.

3.2.2 Growth and selection of isolates to be used for inoculation

Colletotrichum acutatum species complex (referred to as *Colletotrichum acutatum* for the rest of the chapter) and *C. gloeosporioides* species complex (referred to as *Colletotrichum gloeosporioides* for the rest of the chapter) isolates that were identified from the results of Chapter 2 to produce the most spores and achieve the fastest growth were selected for inoculation. These isolates were then evaluated for pathogenicity on´ Blue Lake runner' bean plants. In total, 3 isolates were selected, one *C. acutatum* isolate (C.a4) and two *C. gloeosporioides* isolates (C.g1 and C.g3). The isolates were stored in glycerol at -80^{o}C and sub-cultured onto full strength potato dextrose agar (PDA; Difco Laboratories, MI, USA) in 90 cm Petri dishes. Each isolate was inoculated on 10 plates. Petri dishes were sealed with parafilm and incubated at 25°C under 12/12 photoperiod for two weeks. Conidia were recovered from the Petri plates growing each of the isolate plates and used to produce three different spore concentrations, $1x10^2$, $1x10^4$, and $1x10^6$ conidia/mL for inoculation of the common bean plants in this experiment (Giblin *et al.,* 2010).

3.2.3 Preparation of conidial suspensions for pathogenicity testing.

For each *C. acutatum* and *C. gloeosporioides* isolate a conidial suspension was prepared by adding approx. 10 mL of sterile distilled water plus Tween 20 (Lab Chem, USA; 1 drop in 50 mL water) to each

plate and dislodging conidia from the mycelia using a sterile wire loop. The resulting suspensions were subsequently sieved using cheesecloth into a beaker to remove any mycelial fragments. For each isolate, the conidial concentration in the resulting suspensions was determined by hemocytometer counts and then adjusted to the required concentrations (10^2, 10^4, and 10^6 conidia/mL) using sterile water plus Tween 20. The surfactant, Tween 20 was added to both ensure the dispersal of the conidia in the solution and to facilitate infection.

3.2.4 Inoculation of common bean plants

The common bean plants were inoculated at the onset of the flowering stage (after 5 weeks of growth) when they had approximately 9 fully developed leaves. Inoculation was achieved using a manual atomizer by spraying the whole plant to run off with one of the three conidial suspensions (10^2, 10^4, and 10^6 conidia/mL) for each of the isolates. Each isolate was applied at each of the three conidial concentrations, representing the different inoculation treatments. There were three control treatments in total, plants sprayed with water only, plants sprayed with water solution amended with Tween 20, and plants that were left unsprayed. The twelve treatments were each replicated ten times. To induce conidial germination and maximum infection, the plants were covered with plastic bags (90 cm) which were secured around the pot with an elastic band for 48 hr to maintain 100% relative humidity. The plants were then placed on a bench in the greenhouse in a completely randomized block design. The plants were watered daily using a hosepipe. Water content was maintained at field capacity based on moisture meter readings as described in previous Section 3.2.1. The experiment was maintained until the *P. vulgaris* plants attained podding stage, approximately 28 days after inoculation. Thrips were observed to cause damage to some of the plants 7 days after inoculation and the plants were moved further apart to try and prevent cross-contamination. No insecticides were applied due to the possible effect on *Colletotrichum* infection.

3.2.5 Assessment of disease incidence and severity

Plants were observed on a daily basis for any symptoms. Disease incidence on the leaves was determined at 3, 7, 14 days after inoculation (d.a.i.), pods were assessed 14 days after pod's development (i.e., 28 d.a.i.) for each replicate. Disease incidence was determined by recording the number of leaves per plant showing distortion or chlorosis and the number of pods per plant presenting whitish spots. These were then divided by the total number of leaves and pods on the plant to determine the percentage of infection:

$$(\%\ infection = \frac{No.\ of\ infected\ leaves/pods}{Total\ No.\ of\ leaves/pods} x\ 100).$$

Disease severity was determined using a 0-5 scale described by (dos Santos *et al.*, 2009) where 0 = no visible sign, 1 = 0-20% of leaf/pod area showed sign/infected, 2 = 20-40% of leaf/pod area infected, 3 = 40-60% of leaf area infected, 4 = 60-80% of leaf area infected, and 5 = 80-100% of leaf/pod area infected/plant is dead (Appendix B, Figure B.2). The assessment was based on the overall percentage of infected leaves and pods, and this was done at 14 and 28 d.a.i. for leaves and pods, respectively.

Table 3. 1 A 0-5 scale was used to assess the severity of symptoms of isolates on common bean.

Score	Severity
0	No visible sign.
1	0-20% of leaf/pod area showed sign/infected.
2	21-40% of leaf/pod area infected.
3	41-60% of leaf area infected.
4	61-80% of leaf area infected.
5	81-100% of leaf/pod area infected/plant is dead.

3.2.6 Recovery of the pathogen from infected leaves and pods

To confirm the causal agents, asymptomatic and symptomatic leaves and pods were harvested from the plants for each treatment 14 and 28 d.a.i., respectively using secateurs. For both asymptomatic and symptomatic tissues, one leaf and one pod were identified and harvested from each treatment ensuring no direct contact between the asymptomatic and symptomatic samples. In total, 48 samples (24 asymptomatic leaves and pods; and 24 symptomatic leaves and pods) were acquired. The samples were surface sterilized using 0.1% bleach (NaOCL) and 70% ethanol for 1 min each, rinsed 3 times in sterile water, and dried by placing on clean paper towels in a sterile airflow in a laminar flow hood. For the symptomatic leaves and pods, samples were observed under a dissecting microscope, and two infected areas were marked with molten agar. Two 3 mm diameter sections were then cut from the edge of the marked section of infected tissue (taking one from each of the 2 lesions) using a sterile scalpel blade and placed on one side on the surface of a PDA amended with streptomycin (5 mg /mL; Sigma Aldrich) and penicillin (5 mg /mL; Sigma Aldrich). For asymptomatic samples, two 3 mm diameter sections of tissue were cut from any part of the leaf or pod and placed on the other side of the same PDA plate as the corresponding symptomatic tissue to enable any colonies growing from the tissues to be compared

(Figure 3.10). All plates were sealed with parafilm, arranged in a completely randomized block design, and incubated at 25°C under 12/12 photoperiod for 14 days.

3.2.7 Morphological identification of *Colletotrichum* species

To identify the colonies recovered from the symptomatic and asymptomatic tissue samples, the colonies were sub-cultured onto fresh PDA plates. From each colony that grew from the tissue samples, a mycelial colonized agar disc was taken from the margin of the colony and used to centrally inoculate a fresh PDA plate and further incubated for 14 days under 25°C. Morphological characteristics including colony color, mycelial growth rate, conidial shape, and size were used for the identification of the colonies as either *C. acutatum* or *C. gloeosporioides*. Colony color was observed at 7 days, and growth diameter was recorded at 7 and 14 days on two perpendicular lines marked on the base of the Petri dishes, and then used to determine the colony growth rate. The shape and size (length and width) of the conidia were determined at 7 days after mounting mycelia onto a slide and staining with clear lactic acid (0.5 µL) and lactophenol cotton blue (0.5 µL).

All isolates were placed into 3 groups based on their morphological characteristics. Isolates that produced colonies with white to pinkish color, fusiform shaped conidia, and of 8.5-10.0 µm in length and 4.5-6.0 µm in width were morphologically identified as *C. acutatum* (Sutton, 1992). Isolates which presented with yellow to grayish colony color, ovoid to oblong-shaped conidia 10.0 – 15.0 µm in length and 5.0-7.0 µm in width were morphologically characterized as *C. gloeosporioides*. Isolates that were not morphologically identified as either *C. acutatum* or *C. gloeosporioides* were placed in group 3 and were not identified further. The morphology of the isolates for each treatment was recorded and the morphologies were compared with those of the isolates originally used for inoculation.

3.2.8 Molecular identification of recovered isolates.

To confirm the identity of the isolates recovered from asymptomatic tissue and symptomatic lesions on *P. vulgaris* leaf and pod tissue, sequencing of the ITS gene region and amplification of a single PCR product of the expected size using both *C. acutatum* and *C. gloeosporioides* species-specific PCR was carried out. Three colonies were randomly selected using a random number generator from both the colonies morphologically identified as *C. acutatum* and those identified as *C. gloeosporioides* and cultured on PDA.

Mycelia were harvested from 14-day-old PDA cultures and DNA was extracted as previously described in Section 2.2.4, PCR amplification and sequencing of the ITS gene region were carried out as described in

Chapter 2, Sections 2.2.4.1 and 2.2.4.2. Briefly, universal primer ITS1/ITS4 was used to amplify products by PCR. For the species-specific PCR, DNA from the isolates was amplified using CaInt2/ITS4 specific for *C. acutatum* (5'-GGGGAAGCCTCTCGCGG-3') and CgInt/ITS4 specific for *C. gloeosporioides* (5'-GGGGAAGCCTCTCGCGG-3') (Shi *et al.*, 2008). PCR mix for both ITS primers and species-specific primers pairs were carried out separately in 20 µL reaction volumes. The PCR mix contained 10 µL (1x) DreamTaq™ (Thermo Scientific™) DNA Polymerase with green buffer, 2 µL of 10-60 ng/µL of genomic DNA of each isolate: 1 µL of each primer (ITS1 and ITS4), and 6 µL of molecular water. Water was used as a negative control, in which 2 µL was added to an equal volume of the master mix instead of a DNA fragment.

The PCR was performed using a T100 Thermal Cycler (BIO-RAD, CA, USA), and the conditions for all samples were: Initial denaturation at 95^{o}C for 3 min, followed by 35 cycles of denaturation at 95^{o}C for 30 s, annealing at 54^{o}C for 30 s and extension at 72^{o}C for 1 min and finally 72^{o}C for 7 min. Products were separated on a 1% agarose, and stained with GelRed® (Biotium, USA). Gels were then viewed under UV light using the Versa Doc imaging system (Mode 3000, Bio-Rad, CA, USA), where a single bright band produced by each sample indicated successful amplification, with the 1 kb plus DNA ladder used to determine the expected base pair (bp). The production of a product of approximately 600 bp for primer pair ITS1/ITS4, 496 bp for the *C. acutatum*-specific PCR (CaInt2/ITS4), and 450 - 463 bp for the *C. gloeosporioides*-specific PCR (CgInt/ITS4) was used to identify species (Archana *et al.*, 2018; Shi *et al.*, 2008).

The PCR products from the PCR amplification of the ITS gene region were sequenced at the Lincoln University Sequencing facility using the Applied Biosystem™ (ABI) Prism 3130x1 Gene-Analyzer. The resulting sequences and chromatographs were edited using BioEdit™ sequence alignment editor 7.2. A pairwise local alignment was done with forward and reverse sequences to create consensus sequences that were submitted to GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the basic local alignment search tool (BLASTN) function to determine the *Colletotrichum* species identification. To confirm the identity of the isolates used matched with the original isolates used to inoculate the plants, the nucleotide sequences of the recovered isolates were compared with that of the original isolates using BLASTN.

3.2.9 Statistical analysis

Percentage disease incidence data on leaves and pods were arcsine transformed prior to analysis by two-way Analysis of Variance (ANOVA) using GenStat (version 2019) to determine any significant

difference between treatments, isolates, and treatment interactions on the mean disease incidence. The Bonferroni test at 95% confidence interval was performed to determine which parameter differed significantly from the others. After analysis, the transformed data were back-transformed into percentages for interpretation and presentation using bar charts. Disease score data as a measure of disease severity was analyzed using ANOVA to compare the mean score between inoculum concentrations, and isolate treatment interactions. Bonferroni test was carried out to determine which treatments varied significantly. Data on colony colour, conidia shape, colony growth rate, and conidia length and width were presented using descriptive statistics in a tabular form.

3.3 Results

3.3.1 Disease incidence on leaves

All three *Colletotrichum* sp. isolates tested were infective on *P. vulgaris*, with inoculation concentration of $1x10^6$ conidia/mL of all the three isolates resulting in the most severe infection (Figure 3.2 B-D). The negative control-treated plants also showed very mild symptoms (Figure 3.2 A) observed as mild chlorosis and spotting on the leaves. On all the *Colletotrichum* species inoculated plants, the symptoms began to appear 3 d.a.i. and became severe 10 d.a.i. onwards. Severe leaf distortion and chlorosis of the whole leaf were symptoms consistent in plants inoculated with *C. acutatum* isolate C.a4 (Figure 3.3 C). For plants inoculated with *C. gloeosporioides* isolates C.g1 and C.g3, symptoms consisted of early chlorotic lesions along the midrib and leaf veins which progressed to yellow or chlorotic spots randomly distributed to cover the entire leaf blade (Figure 3. 3 A and B). The affected leaves turned yellow, with leaflets falling off 14 d.a.i. There was no significant interaction (P=0.097) (Appendix B, Table, B.3) between *Colletotrichum* species isolate and inoculum concentration on the disease incidence. There was a significant effect (P=0.012) (Appendix B, Table B.5) of isolate on the infection of leaves with *C. gloeosporioides* isolate C.g3 resulting in significantly higher mean disease incidence (90%) than that with *C. acutatum* isolate C.a4 (79%), *C. gloeosporioides* isolate C.g1 (79%), and the negative controls (12%). Inoculation with *C. acutatum* isolates C.a4 and *C. gloeosporioides* isolate C.g1 resulted in significantly higher mean disease incidence compared with the control treatments (Figure 3.4). Inoculum concentration also significantly affected (P=0.001) (Appendix B, Table B.5) the mean disease incidence. Across all isolates, inoculation with inoculum concentrations $1x10^6$ conidia/mL (90%), $1x10^4$ conidia/mL (70%), and 1×10^2 conidia/mL (75%) resulted in significantly higher mean disease incidence than the no pathogen controls (12%).

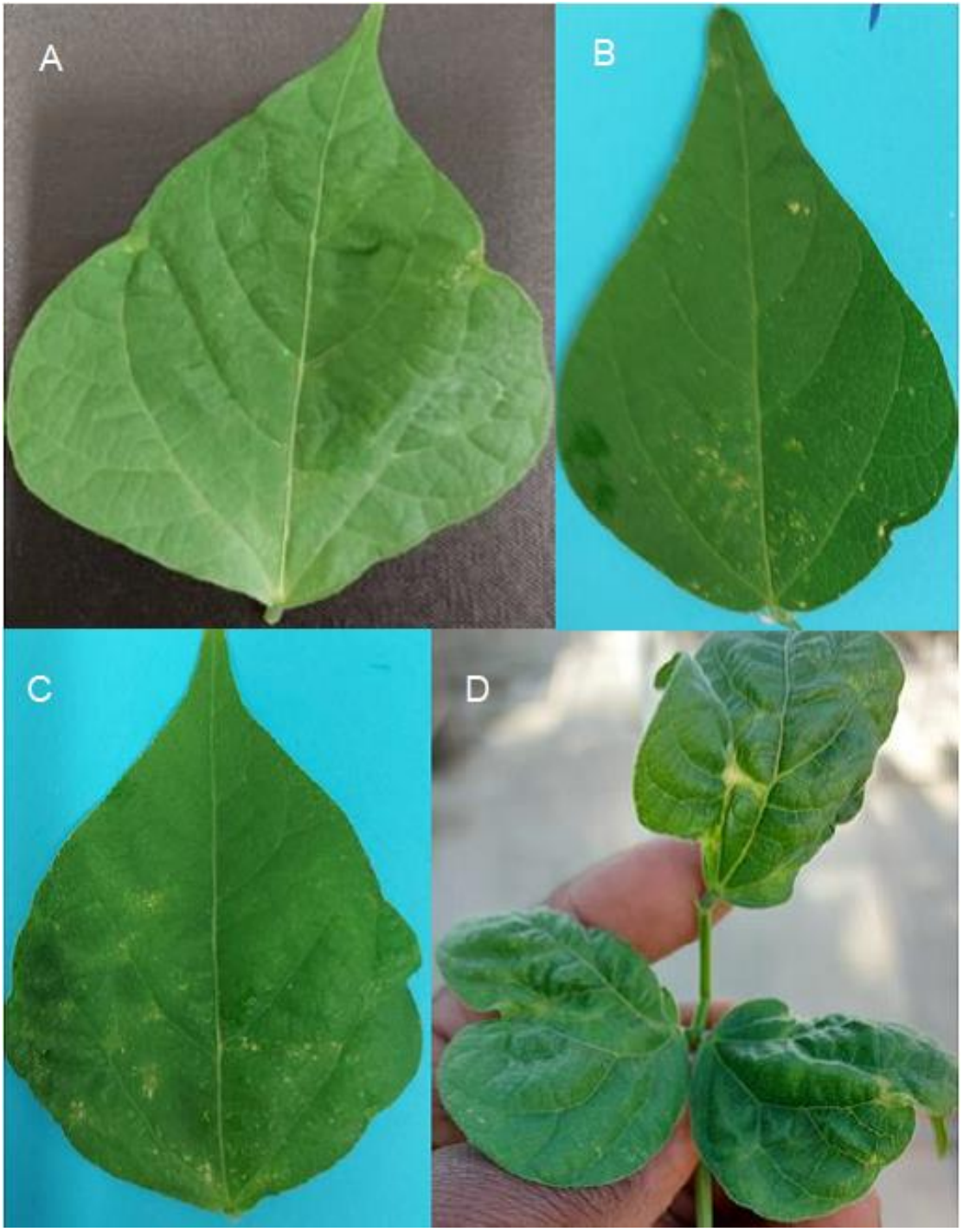

Figure 3. 2 Typical symptoms observed on leaves of *Phaseolus vulgaris* plants inoculated with different concentrations of *Colletotrichum acutatum* or *C. gloeosporioides* conidia 14 days after inoculation. (A) Mild chlorotic flecks produced in the negative controls; (B) and (C) moderate symptoms caused by inoculation with $1x10^2$ conidia/ mL and $1x10^4$ conidia/ mL); (D) severe lesions induced by inoculation with $1x10^6$ conidia/mL).

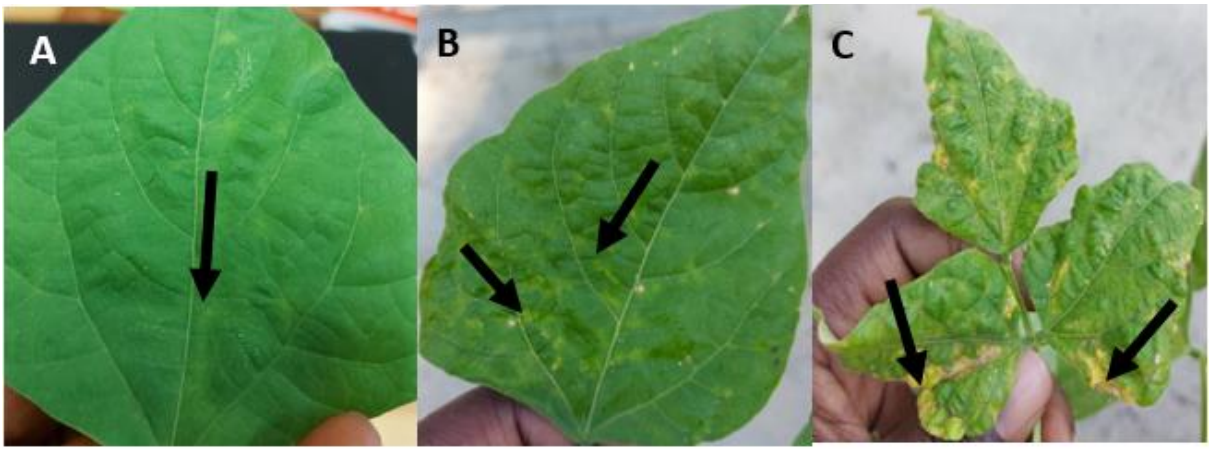

Figure 3. 3 Symptoms observed on *Phaseolus vulgaris* leaves at 3, 7 and 14 days after inoculation (d.a.i) with *Colletotrichum acutatum* or *C. gloeosporioides*. (A) Leaf showing typical symptoms observed following inoculation with *C. gloeosporioides* isolates C.g1 and C.g3, 3 d.a.i. Yellow or chlorotic spots randomly distributed on the leaf; (B) typical chlorotic symptoms caused by *C. gloeosporioides* isolate C.g1 or C.g3 observed along the midrib and leaf veins 7 d.a.i., and (C) severe leaf distortion and intense chlorosis of the whole leaf caused by *Colletotrichum acutatum* isolate C.a4 14 d.a.i. Arrows show sites of infection.

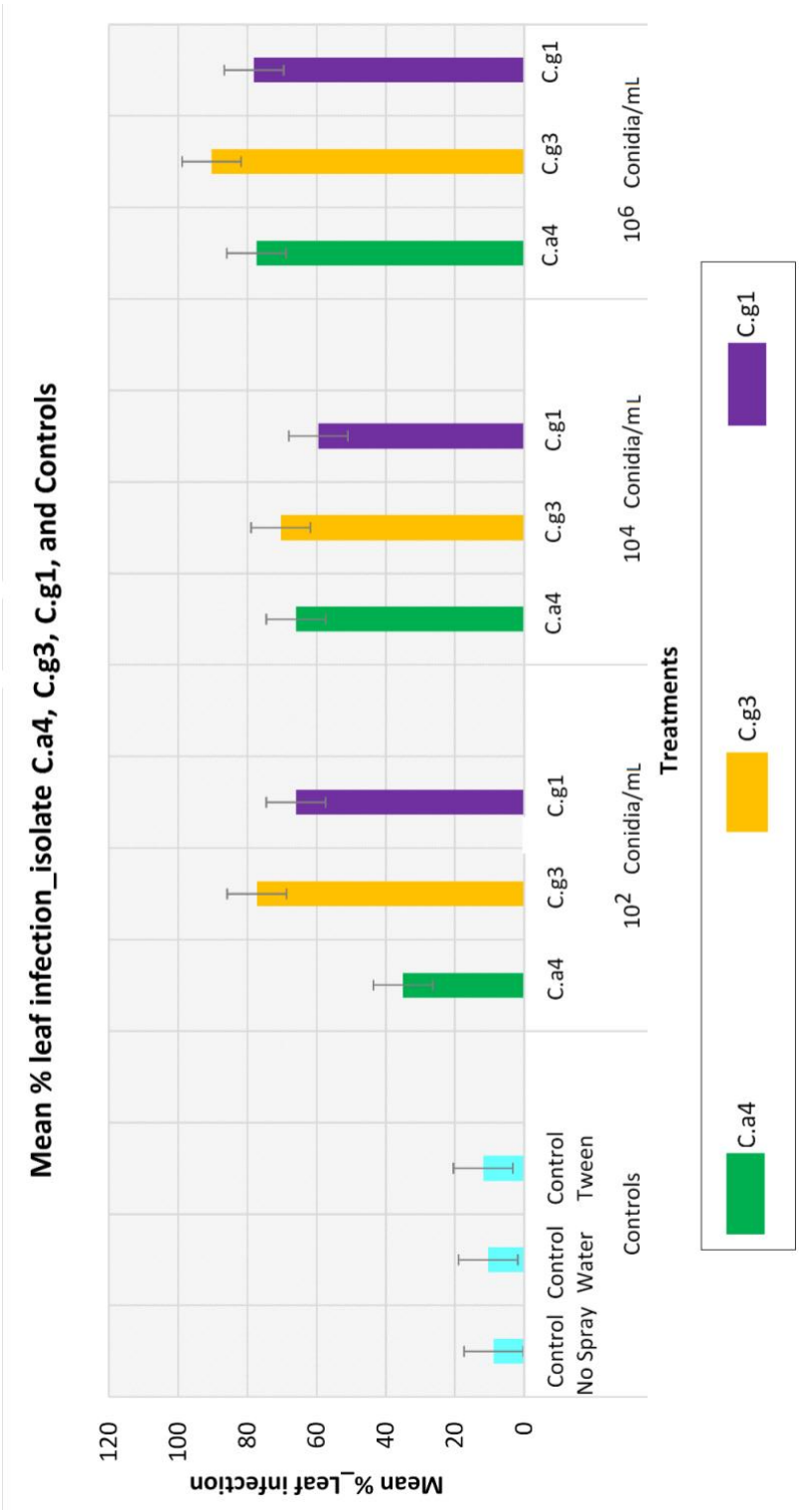

Figure 3. 4 Mean disease incidence (%) on leaves of common bean plants 14 days after inoculation (d.a.i.) with different conidial concentrations of *Colletotrichum acutatum* isolate C.a4 and *C. gloeosporioides* isolates C.g1 and C.g3 compared with the three control treatments (no spray, sprayed with water and sprayed with tween solution). Error bars represent standard error means.

3.3.2 Disease incidence on pods

All three *Colletotrichum* species isolates produced lesions on the pods. Lesions were observed on pods 14 days after the pod's development (i.e., 28 d.a.i.). Small greyish distinct circular lesions were observed as the pods matured (Figure 3.5). Lesions on the pods which developed on the negative control plants were fewer but looked the same as the ones on the *Colletotrichum* inoculated plants. There was no significant interaction (P=0.551) (Appendix B, Table B.4) between *Colletotrichum* species isolate and inoculum concentration (Figure 3.6). Across all *Colletotrichum* species isolates, there was significant effect (P=0.001) of inoculum concentration on the mean disease incidence, with all inoculum concentrations, $1x10^6$ conidia/mL (61%), $1x10^4$ conidia/mL (47%), and $1x10^2$ conidia/mL (48 %), causing higher mean disease incidence than the no pathogen controls (21%) (Appendix B, Table B.6). There was no significant difference (P=0.422) (Appendix B, Table B.5) in the mean disease incidence between the three *Colletotrichum* species isolates, with mean infection being 53.3%, 53.7%, and 54.2% for *C. acutatum* isolate C.a4, *C. gloeosporioides* isolate C.g1, and *C. gloeosporioides* isolate C.g3, respectively.

Figure 3. 5 Typical disease symptoms observed on *Phaseolus vulgaris* pods, 14 days after inoculation with *Colletotrichum acutatum* or *C. gloeosporioides* isolates. Black arrows show the lesion's development.

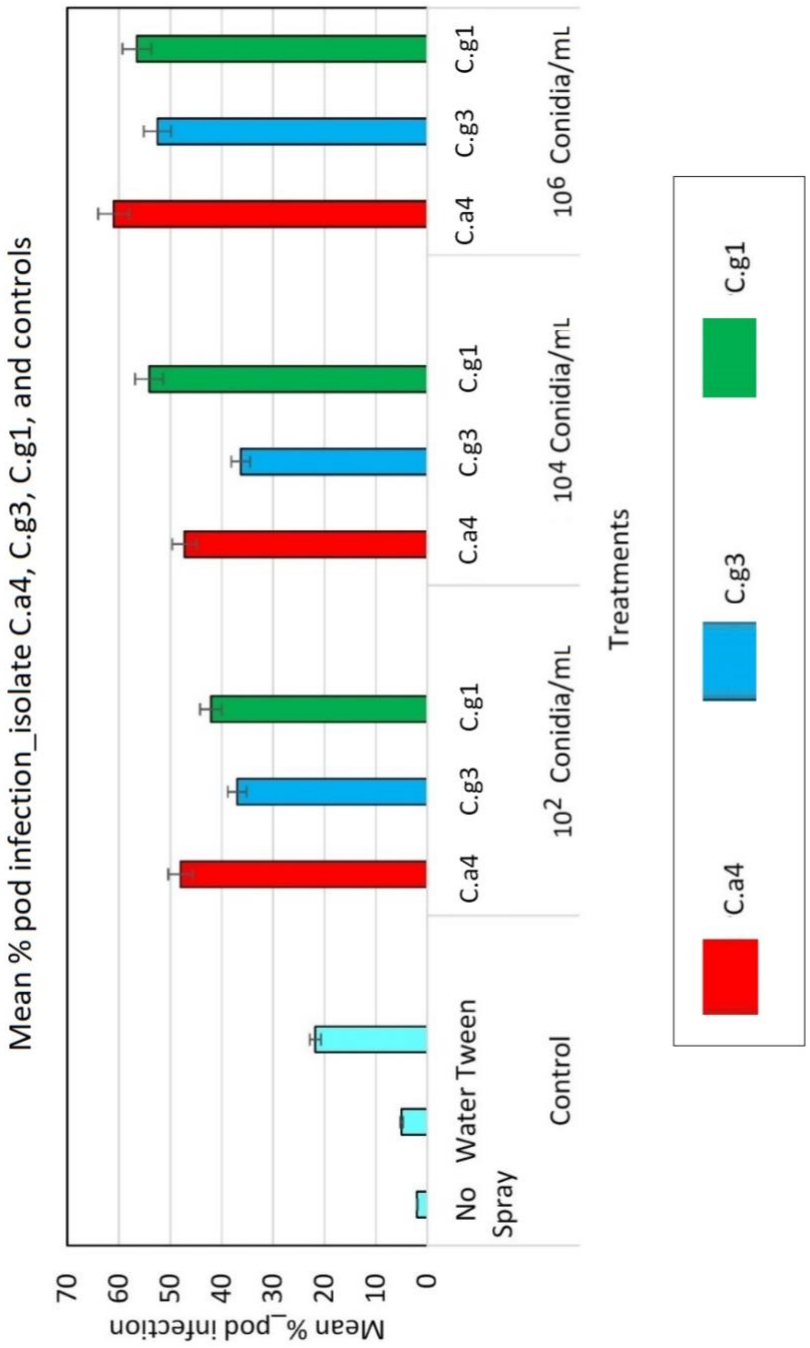

Figure 3. 6 Mean disease incidence (%) of pods on common bean plants 28 days after inoculation (d.a.i.) with different conidial concentrations of *Colletotrichum gloeosporioides* isolates C.g1 and C.g3, and *C. acutatum* isolates C.a4 compared with the three control treatments (no spray, sprayed with water and sprayed with tween solution). Error bars represent standard error means.

3.3.3 Disease severity on whole plants

In addition to the symptoms recorded on leaves and pods, red-brown spots and streaks developed on the stems and petioles of the inoculated *P. vulgaris* plants. Some of the severely infected plants (10.8%) failed to produce pods completely and 4.1% died over the experimental period (Table 3.2). These were mostly for plants inoculated with *C. gloeosporioides* isolates C.g3 (4 plants) and C.g1 (3 plants) at $1x10^6$ inoculum concentration. Dead plants and those that failed to produce pods, were recorded as missing data for the analysis. There was no significant interaction (p=0.084; Appendix B, Table B.6) between isolate and inoculum concentration with the mean disease score for *C. acutatum* isolate C.a4, *C. gloeosporioides* isolates C.g3, and C.g1 being 1.3, 3.0, and 2.8 at concentration $1x10^2$; 3.3, 3.5, and 3.2 at concentration $1x10^4$; 3.8, 4.2, and 3.6 at concentration $1x10^6$, respectively (Figure 3.7). Disease severity was not significantly different (P=0.141; Appendix B Table B5) between *Colletotrichum* species isolates, with the mean disease score for *C. acutatum* isolate C.a4, *C. gloeosporioides* isolates C.g3, and C.g1 being 2.8, 3.6, and 3.2, respectively. There was a significant difference (P=0.001; Appendix B, Table B.5) in the mean disease severity between the inoculum concentrations, with all inoculum concentrations $1x10^6$ conidia/mL (disease score 3.7), $1x10^4$ conidia/mL (disease score 3.4), and $1x10^2$conidia/mL (disease score 3.2) causing significantly higher disease score compared with the no pathogen controls (disease score 1.5).

Table 3. 2 The number and percentage of dead common bean plants and those that did not produce pods across all inoculation treatments were assessed at different days after inoculation (d.a.i).

Days after inoculation (d.a.i)	**No. dead out of 120 plants**	**% Dead plants**	**No. of plants without pods out of 120**	**% Without pods**
3	0	0	0	00
7	0	0	0	0
14	3	1.6	1	3.3
28	4	2.5	3	7.5
Total	7	4.1	5	10.8

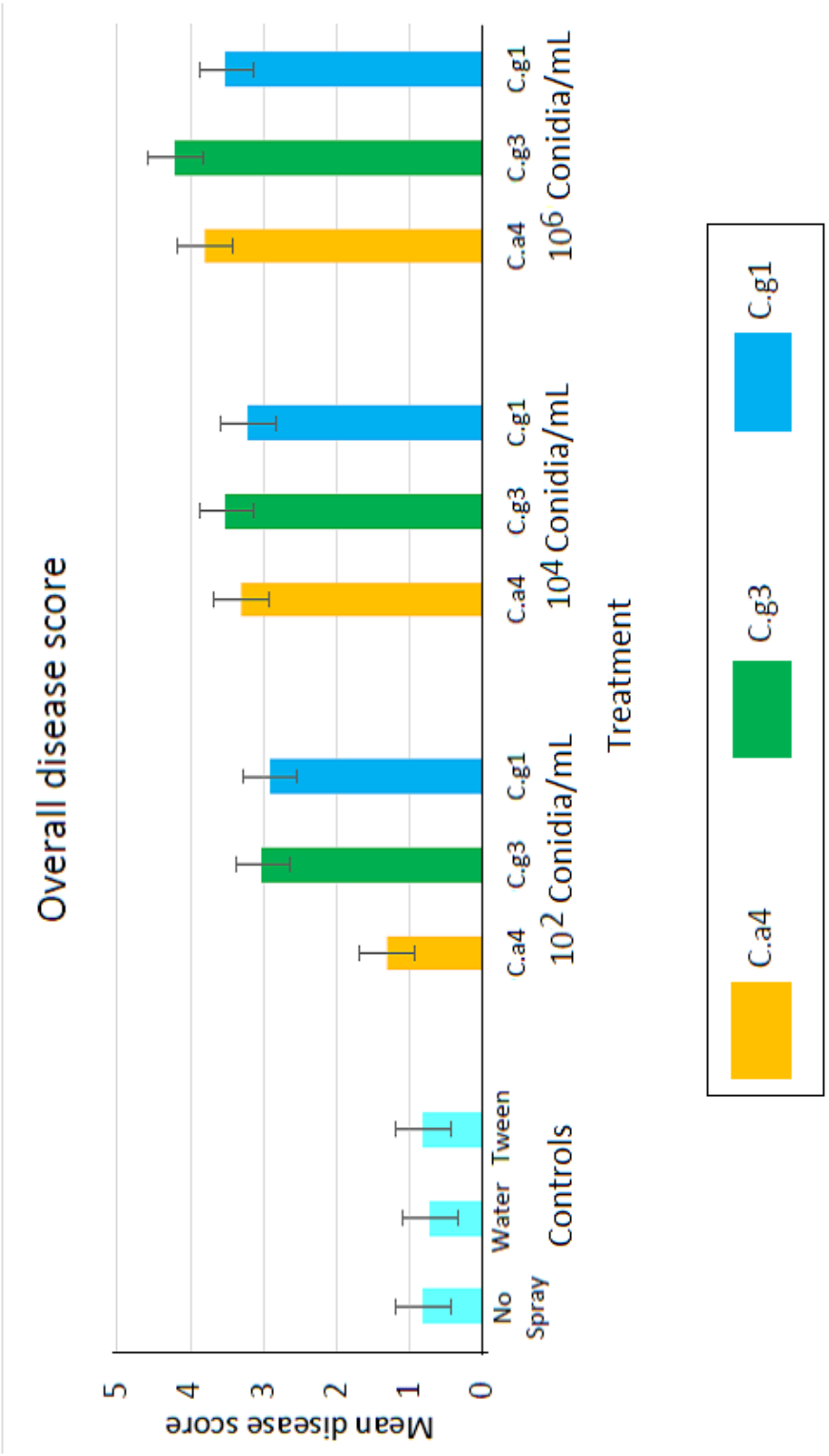

Figure 3. 7 Mean disease score on plants 28 days after inoculation (d.a.i) with different concentrations of *C. acutatum* isolate C.a4 and *C. gloeosporioides* isolates C.g3 and C.g1 compared with the three control treatments (no spray, sprayed with water and sprayed with tween solution). Error bars represent standard error means.

3.3.4 Morphological identification of isolates recovered from diseased leaves and pods

No colonies were recovered from any of the asymptomatic leaves and pods when plated on PDA (Figure 3.8 A1, A2, B1, and B2). Tissue pieces taken from symptomatic samples of plants inoculated with *C. acutatum* isolate C.a4 produced colonies with a pink mass of acervuline on agar. Colonies were white to pinkish in color on both the upper and reverse sides (Figure 3.10 A and B) with sparse aerial mycelium. Conidia were fusiform in shape, medially constricted, measuring 12.0 - 16.5 µm in length and 3.5 - 6.0 µm in width (Figure 3.9 A). These features were consistent with the morphology of *C. acutatum* reported by Sutton (1992) and that of the isolate used for inoculation in Section 3.2.4, and therefore the colonies were morphologically identified as *C. acutatum*. Colonies recovered from symptomatic tissue from plants inoculated with *C. gloeosporioides* isolates C.g1 and C.g3 were smoky greyish in color (Figure 3.10 C). Conidia produced were cylindrical, ovoid to oblong, slightly curved or dumbbell-shaped, hyaline, and measuring 10.0 – 15.0 µm in length and 5.0 - 7.0 µm in width (Figure 3.9 B). These observed characteristics conform to the morphological description of *C. gloeosporioides* reported by Chakraborty *et al.* (2019a); (Meshram *et al.*, 2014) and that of the isolates used for inoculation in Section 3.2.4, and therefore the colonies were morphologically identified as *C. gloeosporioides*. Isolates recovered from the symptomatic lesions on the control plants produced colonies morphologically similar to *C. acutatum* and *C. gloeosporioides*, indicating cross-infection.

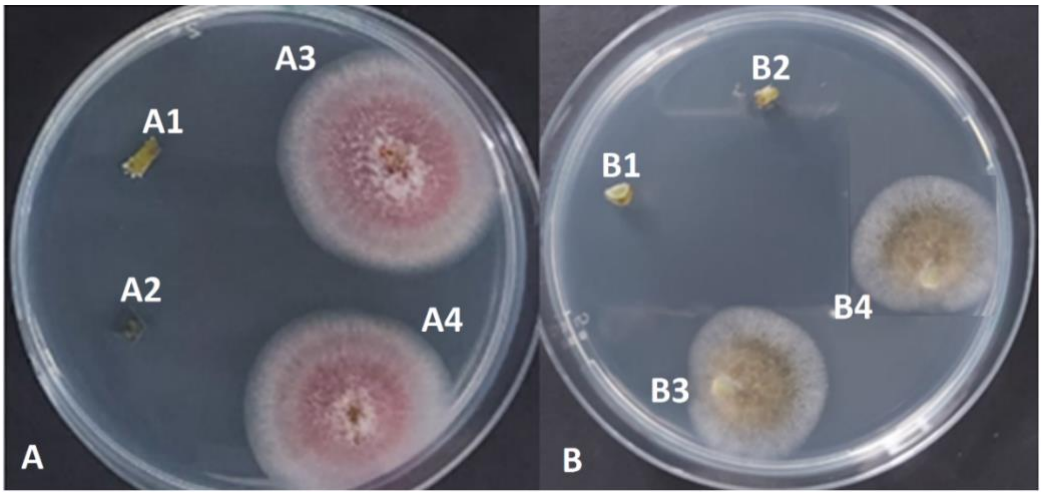

Figure 3. 8 Asymptomatic and symptomatic *Phaseolus vulgaris* leaf and pod tissues plated on PDA. A1 and A2 tissues from asymptomatic leaves of plants inoculated with *Colletotrichum acutatum* isolate C.a4 showing no fungal growth; A3 and A4 tissues from symptomatic leaves of plants inoculated with *C. acutatum* isolate C.a4 showing typical colonies of *C. acutatum* growing on the agar. B1 and B2 tissues from asymptomatic pods of plants inoculated with *C. gloeosporioides* isolate C.g3 showing no pathogen growth on PDA; B3 and B4 tissues from symptomatic pods of plants inoculated with conidial suspension of *C. gloeosporioides* isolate C.g3 with a colony morphologically identified as *C. gloeosporioides* growing from both tissues.

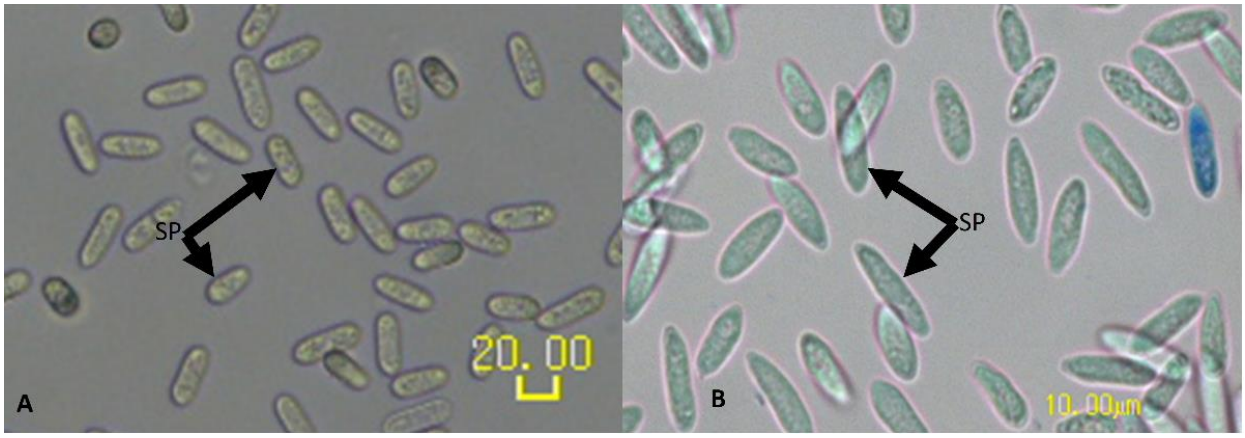

Figure 3. 9 Microscopic images showing conidia produced by isolates recovered from lesions produce on a bean leaf from plants inoculated with A) *C. acutatum* isolates C.a4, and B) *C. gloeosporioides* isolate C.g*3*. Key: SP = Spore.

Figure 3. 10 Colony appearance of isolates which grew out of the symptomatic common bean tissue pieces plated onto agar plates for the different *Colletotrichum* species treatments. Colony recovered from a plant treated with *Colletotrichum acutatum* isolate C.a4 (A) colony upper side, (B) colony reverse side; (C) colonies recovered from a plant treated with *Colletotrichum gloeosporioides* C.g3, upper side.

3.3.5 Molecular identification of pathogens recovered from infected leaves.

DNA amplification with the universal ITS primer pairs ITS1/ITS4 produced a PCR product of similar size (560 bp; Figure 3.12A) for all the recovered isolates. Sequences of the PCR product from the selected isolates (BLPC.a2, BLPC.a4, and BLPC.a5) recovered from the symptomatic tissue of plants inoculated with *C. acutatum* isolate, C.a4 and morphologically identified as *C. acutatum*, had 98.4% and 100% identity, respectively to *C. acutatum* and *C. fioriniae* sequences obtained from the NCBI database (Table 3.3). Since *C. fioriniae* is part of the *C. acutatum* species complex, the ITS gene region available on GenBank is not sufficient to resolve these two species (Barquero Quirós *et al.*, 2013; Ciampi-Guillardi *et al.*, 2014; De Silva *et al.*, 2017a). When the sequences obtained for the recovered isolates were compared with the sequences of the pure culture isolate used to inoculate the plants (C.a4) obtained in Chapter 2, they were 100% identical (Appendix B, Table B.7). Similarly, two isolates, BLPC.g1, BLPC.g2, recovered from the symptomatic tissue of plants inoculated with *C. gloeosporioides* isolates C.g1, and

one isolate BLPC.g3, from C.g3, morphologically identified as *C. gloeosporioides* had 99.8, 100, and 100% identity, respectively to *C. kahawae* and *C. gloeosporioides* sequences obtained from the NCBI database (Table 3.3). Comparably, since *C. kahawae* is part of the *C. gloeosporioides* species complex, the ITS gene region sequence is not sufficient to accurately identify species within this complex (Weir *et al.*, 2012c). Also, when the sequences obtained for the recovered isolates were compared with the sequences of the pure culture isolates (C.g1 and C.g3) used to inoculate the plants obtained in Chapter 2, they were 100% identical (Appendix B, Table B.7).

Table 3. 3 BLAST results of the ITS gene region of Isolates recovered from inoculated plants.

Isolate	**Source**	**Identity**	**Query cover**	**% Identity**	**Accession number**	**Length (bp)**
BLPC.a2	Bean leaf	*Colletotrichum acutatum*	100	100	MG554643.1	589
BLPC.a4	Bean leaf	*Colletotrichum fioriniae*	100	100	MT133292.1	595
		Colletotrichum acutatum	100	98.4	MN856423.1	951
BLPC.a5	Bean leaf	*Colletotrichum fioriniae*	100	100	MT133290.1	596
BLPC.g3	Bean leaf	*Colletotrichum kahawae*	100	99.8	MN273080.1	556
		Colletotrichum aotearoa	100	99.8	MN273068.1	546
BLPC.g2	Bean leaf	*Colletotrichum gloeosporioides*	100	100	MT083978.1	593
		Colletotrichum kahawae	100	100	MT133276.1	588
BLPC.g1	Bean pod	*Colletotrichum gloeosporioides*	100	100	MF495411.1	599

Specific primer pairs CgInt/ITS4, yielded the expected 460 bp DNA product only for the three isolates (BLPC.g1, BLPC.g2, and BLPC.g3) recovered from *C. gloeosporioides* inoculated plants; while amplification with primers CaInt2/ITS4 produced the expected 496 bp DNA product only for three isolates (BLPC.a2, BLPC.a4, and BLPC.a5) recovered from *C. acutatum* inoculated plants (Figure 3.11B, and C). Based on these results, the three isolates, BLPC.a2, BLPC.a4, and BLPC.a5, recovered from *C. acutatum* inoculated plants were confirmed to be the *C. acutatum* isolate used to inoculate the bean plants. Similarly, the three isolates BLPC.g1, BLPC.g2, and BLPC.g3 recovered from *C. gloeosporioides* inoculated plants were confirmed to be the *C. gloeosporioides* isolates used to inoculate the bean plants.

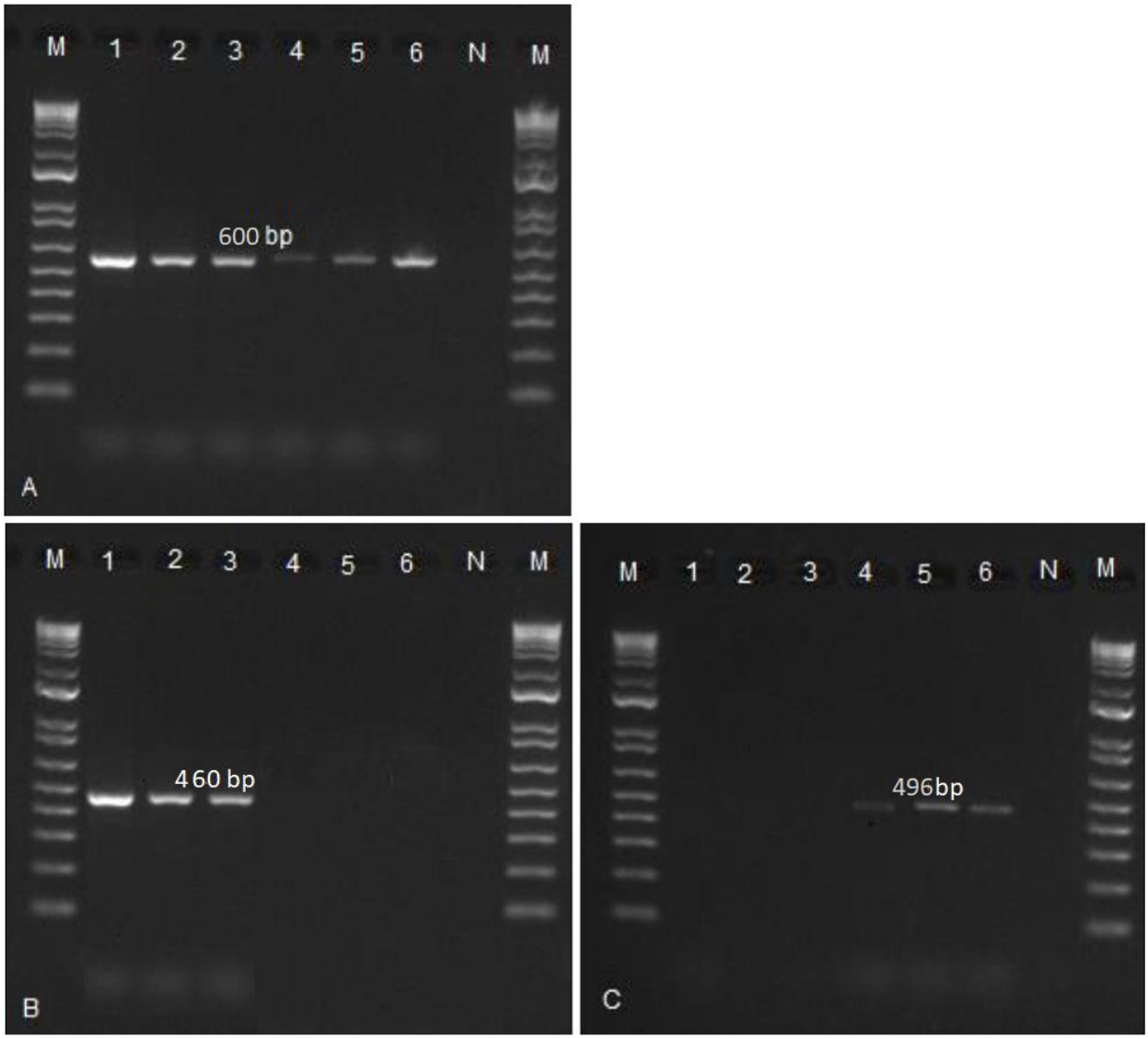

Figure 3. 11 Bands indicate positive amplification of specific DNA fragments for isolates BLPCa2, BLPCa4, BLPCa5, and BLPC.g1, BLPC.g2, and BLPC.g3. (A) Universal primer pairs ITS1/ITS4; (B) Primer pairs CgInt/ITS4 specific for *C. gloeosporioides* showing positive amplification of isolates BLPC.g1, BLPC.g2, and BLPC.g3 (lane 1, 2 and 3; (C) CaInt2/ITS4 specific for *C. acutatum* showing positive amplification of isolates BLPCa2, BLPCa4, and BLPCa5 (lanes 4 to 6); M = molecular size marker (1 kb + ladder); N = negative control (water).

3.4 Discussion

In this study, the pathogenicity of one isolate from the *C. acutatum* species complex (C.a4) and two isolates from the *C. gloeosporioides* species complex (C.g1 and C.g3) applied at varying inoculum concentrations were tested on common bean, *Phaseolus vulgaris,* in the glasshouse. Although the infectivity of *C. gloeosporioides* has been previously reported by Barcelos *et al.* (2014b); (Marcenaro & Valkonen, 2016) this is the first report of an isolate from the *C. acutatum* species complex as a potential pathogen of bean. Results showed that all isolates were infective on common beans. There was a time course progression of typical disease symptoms in response to the fungal pathogen. Fungal colonies re-isolated from symptomatic leaves and pods were similar in morphological characteristics to the original isolates used to inoculate, thus satisfying Koch's postulates. No fungal colonies were recovered from any of the asymptomatic leaves and pods samples. Molecular analysis of representative isolates recovered from the inoculated bean plant tissues also confirmed *C. gloeosporioides* species complex and *C. acutatum* species complex as the causal agents. Although Marcenaro and Valkonen (2016) reported C. *gloeosporioides* as being a seed-borne pathogen of beans that infects beans at the seedlings stage, this study adds to the few literature reports available on the role of *C. gloeosporioides* and *C. acutatum* in causing infection in *P. vulgaris*. In contrast to the current study, Barcelos *et al.* (2014b) reported isolates from the *C. gloeosporioides* species complex to produce only mild symptoms on the inoculated bean. However, the results of this study are consistent with the results of the earlier study by Chakraborty *et al.* (2019b) in which *C. gloeosporioides* isolate produced severe disease symptoms, when used to inoculate bean leaves, with symptoms observed two days after inoculation. The differences in the reports of the potential pathogenicity of *C. gloeosporioides* isolates on common bean is likely to be due to differences in the exact species within the species complex used in these studies. Further work is needed to determine the species within both the *C. gloeosporioides* and *C. acutatum* species complex responsible for mango anthracnose in Uganda and to test the pathogenicity of those particular species on beans.

For all isolates, the three conidial concentrations (10^6, 10^4, and 10^2 conidia/mL) used to inoculate the bean plants resulted in infection *P. vulgaris,* with there being no difference in the disease incidence or disease severity between the different concentrations. In the study by Bertetti *et al.* (2009) a conidial concentration of at least 10^4 conidia/mL was needed to cause infection of Azalea by *C. acutatum,* with concentrations of 10^5 and 10^6 conidia/mL producing the most severe infection. Similarly, Obanor (2006) reported that the number of lesions which developed on olive leaves sprayed with conidial suspensions of the olive leaf spot pathogen *Venturia oleaginea* (as *Spilocaea oleagina*) increased as the inoculum

concentration increased from $1x10^2$ to $2.5x10^5$ conidia/mL. The current results show that for beans, the minimum number of conidia required for infection by the *C. acutatum* and *C. gloeosporioides* species complex isolates used in the study is less than $1x10^2$ spore/ mL. Further, although lesion number per se was not assessed in the current study, the disease severity assessment did partly take this into account, with the results showing no difference in the disease severity caused by the different inoculum concentrations. Since the plants were sprayed with the conidial suspensions it is unclear how many conidia per infection site was required for infection and subsequent lesion development. (Shafi, 2016) reported that for *Neofusicoccum luteum,* infection of grapevines with as few as 2 conidia per wound site resulted in 100% infection incidence of green shoots and 67% incidence of trunks. However, the absence of any difference in the disease incidence between the inoculum concentrations used in the study may be due partly to self-inhibition, with high conidial concentrations of both *C. acutatum* and *C. gloeosporioides* reported to germinate poorly (Kenny *et al.*, 2012; Lax *et al.*, 1985) with maximum germination seen for low conidial concentration. Given that the lowest conidial concentrations of 10^2 conidia/mL tested in the experiment resulted in infection of the bean plants, eliminating all sources of inoculum will play an important role in reducing infection by *Colletotrichum* in these cropping systems.

The ability of *Colletotrichum* isolates originally recovered from avocado and apple fruits to infect another host such as beans demonstrates the potential of these pathogens to attack a wide range of hosts. Research indicates that most crops are susceptible to more than one species of *Colletotrichum* and *Phaseolus vulgaris* is one such crop as found in the present study. Various *Colletotrichum* species such as *C. acutatum, C. capsici, C. coccodes, C. falcatum, C. gloeosporioides, C. sansevieriae,* and *C. truncatum* have been shown to cause severe damage to different plants under different environmental conditions (Dean *et al.*, 2012; Weir *et al.*, 2012b). Zakaria *et al.* (2000) also reported that *Colletotrichum* species isolated from leaves of tropical forest trees were highly pathogenic on common beans. This illustrates the risk of *Colletotrichum* species pathogenic to one crop to cause disease on other crop hosts which are co-cultivated, as is the case of the mixed cropping systems common in Uganda.

The appearance of symptoms on a few uninoculated control plants was shown to be due to cross-infection as colonies morphologically identified as *C. acutatum* and *C. gloeosporioides* were recovered from these lesions. This cross infection is likely to have occurred during the watering of the plants. Water splash during routine watering coupled with the relatively small distance between the plants is likely to have resulted in the splash dispersal of conidia from the inoculated plants. Further, thrips were observed to cause damage on some of the plants and could have also contributed to the cross-infection

between the treated and the untreated control plants. Insect species have been shown to mechanically transport spores and significantly increase the infection rates of plants (Kluth *et al.*, 2002). To aim to minimize the cross-contamination, the plants were spaced further apart 7 days after inoculation. However, in future experiments overhead watering of the plants should be avoided, and watering should be done via saucers placed under each pot to minimise any possible cross contamination of *Colletotrichum* species inoculum.

Results from the current study showed that one isolate of *C. gloeosporioides* species complex (C.g3) caused higher disease incidence on the bean leaves than the other *C. gloeosporioides* species complex isolate (C.g1) and the one *C. acutatum* species complex isolate (C.a4) tested. However, this difference was not observed in pods or overall disease severity. Since only 3 isolates; one *C. acutatum* and two *C. gloeosporioides* species complex were tested, no conclusions regarding the relative pathogenicity of the different *Colletotrichum* species can be made. Further, the two *C. gloeosporioides* species complex isolates may represent different species within the species complex, although identification of the isolates based on sequences of the ITS gene region of the rDNA (Chapter 2) indicated that both were likely to be the same or at least very closely related species. However, sequencing of other gene regions as discussed in Chapter 2 is needed to confirm this. Chakraborty *et al.* (2019a) reported that the pathogenicity of a *C. gloeosporioides* isolates on *P. vulgaris* was associated with changes in the production of signaling molecules associated with the activation of disease resistance mechanisms in the host plant. This included the downregulation of nitric oxide (NO) production and increased generation of reactive oxygen species (ROS) in the form of H_2O_2 following exposure of the host to the pathogen. Whether different isolates of the same species or different species within these two pathogen complexes vary in their ability to affect the production of these signaling molecules to affect plant susceptibility is unclear. Further research involving additional isolates of known species within these two species complexes is required to determine the relative pathogenicity of these species and isolates within these species.

In this present study, the susceptibility of common bean to *C. gloeosporioides* and *C. acutatum* was only tested on one cultivar, 'Blue Lake Runner'. The pathogenicity of these species on other bean cultivars, including one commonly grown in Uganda, remains unknown. However, similar susceptibility could be possible based on growth habits, especially with the presence of R genes in some cultivars. Gaudencia J. Kiptoo (2020) reported the susceptibility of different bean cultivars commonly grown in Kenya to another species, *Colletotrichum lindemuthianum*, and showed they varied in their susceptibility. Forty

percent of the cultivars tested in their study were potentially tolerant or resistant to *C. lindemuthianum* and thus recorded lower incidences and severity than susceptible genotypes. Similarly, a joint study using cultivars from Uganda and Brazil showed that of the cultivars evaluated, 6 cultivars with known resistance genes, as well as 3 cultivars whose resistance genes were uncharacterized, showed good resistance to *C. lindemuthianum* infection (Awori, 2018). These cultivars were shown to be a potentially important resource for breeding programs designed to produce resistant cultivars. The cultivar used in this trial is known in Uganda as French bean with the commonest type grown in Uganda being pole green bean cultivars. French bean varieties such as 'Blue Lake Runner' and pole green bean varieties are similar in their anatomical characteristics and are therefore likely to be similar in their susceptibility to *Colletotrichum* species. Future research should examine the susceptibility of different cultivars, including those commonly grown in Uganda, to identify potential resistant or tolerant cultivars for both cultivations, and for any subsequent breeding programs.

3.5 Conclusion

This study confirmed the pathogenicity of isolates of *C. gloeosporioides* species complex and determined for the first time the pathogenicity of an isolate of *C. acutatum* species complex on a *Phaseolus vulgaris* cultivar similar to the cultivars grown in Uganda in mango plantations. It further reveals that across the inoculum concentrations used in the study, there was no difference in the disease incidence or severity on either leaves or pods. The study thus provides the basis for developing anthracnose control strategies in a cropping system such as mango where *P. vulgaris* is often used as an under-planted crop. Further research work is needed to examine the transmission mechanisms of the pathogens between the under-planted crops and any already established perennial crop.

Chapter 4. Recovery of *C. acutatum* and *C. gloeosporioides* from *Phaseolus vulgaris* plant debris.

4.1 Introduction

Common bean (*Phaseolus vulgaris L*) is an annual leguminous plant that belongs to the genus, *Phaseolus*, with pinnately compound, large trifoliate leaves. Common bean shows variation in growth habits from determinate bush to indeterminate, climbing types. The bushy type is the most predominant type grown in Africa (Buruchara, 2007). The crop is a major source of dietary protein in Eastern and Southern Africa. Common bean contains high protein content and is a good source of energy, folic acid, dietary fibre, and complex carbohydrates (Edje *et al.*, 1980; Platt, 1962). Although grown mainly for subsistence, approximately 40% of production is sold at a market value of USD 452 million (David *et al.*, 2000; Wortmann, 1998).

Common bean is grown in a range of cropping systems. About 74% of the growing area of common bean in Eastern Africa is cultivated under multiple cropping systems (Wortmann, 1998), mainly in association with maize (*Zea mays*), banana (*Musa* species), root and tuber crops, sorghum (*Sorghum bicolor)* or finger millet (*Eleusine coracana*) (Allen & Edje, 1990). In Uganda, the common bean crop is mainly used to under-plant perennial crops such as mango (*Mangifera indica*), citrus (*Citrus* species), avocado (*Persea americana*), coffee (*Coffea arabica*), and papaya (*Carica papaya*), especially during the early stage of orchard establishment to optimize land use.

Harvesting practices involve uprooting the stalks after leaves have fallen off and the pods are dried. Threshing operations are often done at home or in the field. In all cases, the plant debris infected by pathogens may still remain in the soil until the next growing season. Although in most cases the debris is ploughed into the soil in the subsequent field operations, further ploughing could bring the debris back to the surface and if the pathogens are still viable it can act as an inoculum source. Since *Colletotrichum* species infect common bean and are known to survive on host plant debris, the debris left on the surface of the soil in the field after harvest could serve as a potential primary source of inoculum for anthracnose disease in a mango cropping system.

Colletotrichum species that infect beans have been reported by Tu (1983). Conidia of *C. acutatum* and *C. gloeosporioides* have been shown to survive for up to one year in autoclaved soil, however conidial

viability of isolates of both species was reported to decline rapidly in unsterilized soil (Freeman *et al.*, 2002). Further, the pathogens were recovered from up to 30% of the artificially inoculated mummified strawberry fruit after 5 months of burial in the soil (Freeman *et al.*, 2002). *Colletotrichum gloeosporioides* is reported to remain viable for up to 90 days on infected plant parts buried in the soil and up to 150 days on black pepper tissue kept in paper bags (Kumari, 2002).

Since soil fumigation, which is often done to remove inoculum load, is not a common practice in Uganda, infected annual crop debris could act as a source of inoculum for infecting new or already established crops in subsequent seasons. In a cropping system where annual crops are consistently in association with perennial crops, information about the ecology of *C. acutatum* and *C. gloeosporioides* and potential inoculum reservoirs is thus important in developing effective control strategies for mango anthracnose disease. Despite the economic importance, few studies have investigated the epidemiology of mango anthracnose disease. An improved understanding of how these pathogens survive, the infection processes, and epidemiology will inform the development of more efficient control and management strategies. Thus the purpose of this experiment was to examine the survival of *C. acutatum* and *C. gloeosporioides* on the debris of *P. vulgaris* when left on the soil surface or buried under glasshouse conditions, to determine its potential as a primary inoculum source in a mango orchard.

4.2 Materials and methods

4.2.1 Fungal isolates and inoculum preparation

Colletotrichum acutatum isolates C.a4 and *C. gloeosporioides* isolate C.g3 were selected for this experiment based on the results from the pathogenicity experiment (Chapter 3). The isolates were grown as detailed in Chapter 2, Section 2.2.1. Conidial suspensions were produced as described in Chapter 3, Section 3.2.3.

4.2.2 Growing of the bean plants

Bean seeds (cultivar “Blue Lake Runner”) were planted at a depth of 5 cm (5 seeds/pot) in 2.5 litre plastic pots containing potting mix (recipe in Appendix B1) as described in Chapter 3, Section 3.2.1. One hundred and fifty pots were set up, each with five seeds per pot, giving a total of 750 seedlings. The pots were placed on benches in a glasshouse at the Lincoln University nursery and the plants were grown for 5 weeks until flowering. The plants were watered daily.

4.2.3 Media preparation

The growth medium used was a mixture of field soil and commercial potting mix. The potting mix contained 60% peat moss in combination with composted pine bark, lime, and fertilizer amendments, as per the recipe described in Appendix B1. Templeton loam soil was obtained from a fallow field site at Lincoln University. The soil was taken from the surface to a depth of approximately 25 cm using a shovel. The fresh soil sample was initially processed to break any large clods and then sieved two times to remove any course material using 10 mm and 2 mm sieves, respectively.

4.2.4 Preparation of plant materials

A total of 2400 g of bean plant material was harvested at the flowering stage by cutting the stems at the soil level using secateurs. Half of the material (1200 g) was used for *C. acutatum* isolate C.a4 treatments, and the other half for *C. gloeosporioides* isolates C.g3 treatments. For each of the *Colletotrichum* species isolates, the bean material was then divided into 2 equal 600 g portions. One 600 g bean tissue sample was blended (Figure 4.1 a, b) in a Thermomix blender (Vorwerk^{TM}) for 3 min whilst the other 600 g portion was coarsely cut using secateurs (Figure 4.1 c and d). For each of the 600 g samples these were further divided into 4 equal (150 g) samples with 2 samples from each inoculated with 20 mL conidia suspension of *C. gloeosporioides* isolate C.g3 or *C. acutatum* isolate C.a4 at a concentration of 1 x 10^5 conidia per mL, by spraying using a manual atomizer. This concentration was used because 1 x 10^4 conidia per mL or more caused severe infection in the pathogenicity trial. The other two 150 g samples were left uninoculated as controls (Figure 4.2).

Figure 4. 1 Coarsely cut and blended bean tissue material used in the experiment. a) Coarsely cut bean tissue, and b) blended bean tissue using a Thermomix Blender.

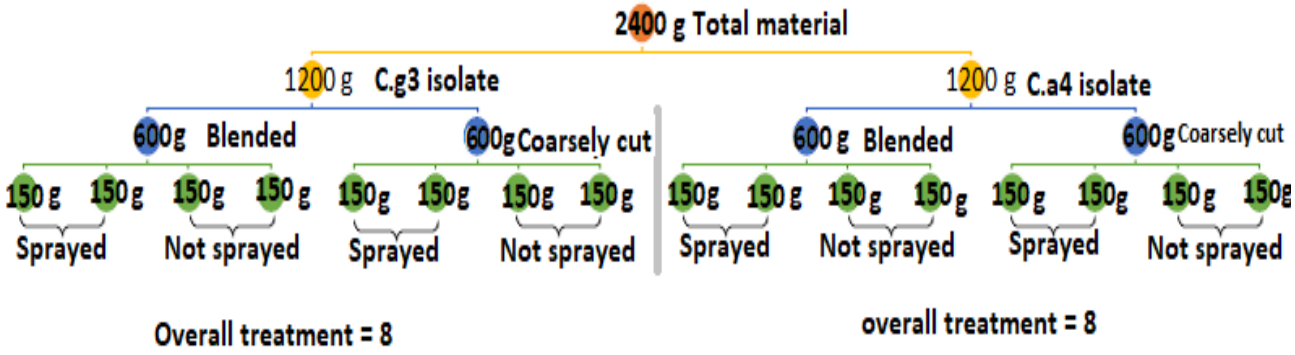

Figure 4. 2 Flow diagram for the bean material preparation for the different treatments, including blended or coarsely cut and sprayed with conidial suspensions of *C. acutatum* isolate C.a4, *C. gloeosporioides* isolate C.g3, or uninoculated (not sprayed).

4.2.5 Experimental design

(Borowik & Wyszkowska, 2016) reported that the survival of fungi was optimum at a soil moisture level of 60% water holding capacity (WHC), hence it was chosen for the experiment. The WHC of the potting mix: soil mixture was determined using the method described by (Rex *et al.*, 2015). Briefly, 100 g of the air-dried potting mix: soil mixture (3 replicates) placed in polypropylene cylinders with the bottom closed with cheesecloth and filter paper in a tray of water for 24 hr to fully saturate. The cylinders containing the potting mix:soil mixture was then left to drain for 24 hr and the weights of the drained saturated soil were recorded. The air-dried potting mix: soil mixture samples were then oven-dried at 105°C for 24 hr, the weight was recorded and used to determine the WHC using the following equation:

$$\text{WHC (\%)} = \frac{(Wet\ potting\ mix{:}\ soil\ mixture\ (g) - Dry\ potting\ mix{:}\ soil\ mixture(g))}{Dry\ potting\ mix{:}\ soil\ mixture\ (g)}\ x\ 100$$

Prior to setting up the experiment, the potting mix: soil mixture was adjusted to 60% WHC (Appendix C, Table C.1). For both *C. gloeosporioides* isolate C.g3 and *C. acutatum* isolate C.a4, eight treatments, each with 10 replicates, were set up: Treatment 1, buried, inoculated blended bean tissue; Treatment 2, surface, inoculated blended bean tissue; Treatment 3, buried inoculated, coarsely cut bean tissue; Treatment 4, surface, inoculated coarsely cut bean tissue; Treatment 5, buried, uninoculated blended bean tissue; Treatment 6, surface, uninoculated blended bean tissue; Treatment 7, buried uninoculated coarsely cut bean tissue, and Treatment 8, surface, uninoculated coarsely cut bean tissue.

For the buried treatments (Treatments 1, 3, 5, and 7) 100 g of the potting mix: soil mixture was placed into plastic pots (0.5 L), and a 10 g layer of inoculated/uninoculated finely blended or coarsely cut bean plant material was placed on top of the mixture. The bean material was then covered with a 5 cm layer of the potting mix: soil mixture. For the surface treatments (Treatments 2, 4, 6, and 8), the pots were filled with 100 g of the potting mix: soil mixture and a 10 g layer of the same bean material were placed on top of the mixture.

The pots were placed in a randomised block design, with 5 blocks placed in each of 2 trays and placed on a bench in a Lincoln University glasshouse (Figure 4.3). The experiments for *C. acutatum* isolate C.a4 and *C. gloeosporioides* isolate C.g3 were set up separately. Each pot was weighed initially and watered daily to the original weight to maintain 60% WHC throughout the experiment. Water was added using a 20 mL hypodermic syringe to ensure even application of water. The experiment ran for 6 weeks to allow degradation of plant material.

Figure 4. 3 Layout of pots with *Colletotrichum acutatum* or *C. gloeosporioides* inoculated or uninoculated bean plant material either placed on the surface or buried in the potting mix: soil mixture in pots to determine their effect on the survival of *Colletotrichum* in plant debris.

4.2.6 Harvest and assessments

4.2.6.1 Harvest

The bean tissue sample material on the surface of the potting mix: soil mixture was recovered and placed into separate new plastic bags. To retrieve the buried bean tissue material samples, the top 5 cm potting mix: soil mixture layer covering the buried material were carefully removed using a spatula. The spatula was cleaned between each replicate and a separate spatula was used for each treatment to avoid cross-contamination. For the buried material, approximately 15 g was sampled, containing

partially decomposed material and adjacent potting mix: soil mixture, and placed in separate new plastic bags.

4.2.7 Soil dilution

4.2.7.1 Pilot assay to determine agar selection

To determine the best media for isolation of *Colletotrichum acutatum* and *C. gloeosporioides* species by serial dilution, five different growth media, $^1/_5$ strength Potato Dextrose Agar (PDA; Becton Dickinson & Co, Spark, USA), $^1/_2$ strength PDA, full-strength PDA, prune agar, and V8 agar was tested. Each of the agar was amended with 10 mg/L of both streptomycin and penicillin to prevent bacterial growth. The media recipes are described in Appendix C, Table C.2.

Before the setup of the main experiment, pots for each of the treatments described in Section 4.2.5 were set up and the crop debris were harvested after 6 weeks and used for dilution plating on each of the media. Samples of the crop debris were taken using a pair of forceps and suspended in 9 mL of SDW, shaken for 10 min on a wrist action shaker before leaving to stand for a further 10 min. The resulting suspension was then used to produce a dilution series of 10^{-4}. A 1 mL aliquot of the final dilution $1x10^{-4}$) was pipetted onto the surface of three replicate plates for each of the media. The suspension was then gently spread over the surface of the agar with a sterile plastic loop and the plates were sealed with parafilm. The plates were incubated at 25^{o}C with a 12/12 hr photoperiod and after 48 hr were observed for the appearance of colonies characteristic of *Colletotrichum* species. The agar medium which recovered the highest number of *Colletotrichum* species colonies with the lowest level of other fungal contaminant colonies was selected for use to recover *Colletotrichum* species in the experiment.

4.2.7.2 Determining background *Colletotrichum* species levels in the potting mix: soil medium

Samples of the bulk potting mix: soil were taken to determine the background incidence of *Colletotrichum acutatum* or *C. gloeosporioides* in the potting mix: soil mixture used to set up the experiment. A total of 80 samples (1 g each) of the bulk potting mix: soil mixture were taken. The samples were individually suspended in 9 mL of sterile distilled water (SDW) and used to produce a dilution series to 10^{-4} as described previously. A 1 mL aliquot of the final dilution ($1x10^{-4}$) was spread plated onto the surface of three replicate plates of prune agar medium. The plates were incubated at 25^{o}C with a 12/12hr photoperiod for 48 hr. The number of colonies morphologically identified as *Colletotrichum* species were counted and the number of colony-forming units per gram (CFU/g) of the original potting mix: soil sample was determined.

4.2.7.3 Evaluation of *Colletotrichum* species incidence on bean tissues at harvest

For each replicate, 15 g of the recovered bean tissue material and any attached soil particles were placed on tissue paper, a representative 1 g sample was taken using a pair of forceps (Figure 4.4) and serially diluted in 9 mL of SDW as described previously. After 48 hr incubation, the number of colonies morphologically identified as *C. acutatum* for the *C. acutatum* treatments, and *C. gloeosporioides* for the *C. gloeosporioides* treatments were counted and used to determine the CFU/g for each replicate. For the control treatments, only plates that showed colony growth were selected for CFU counting.

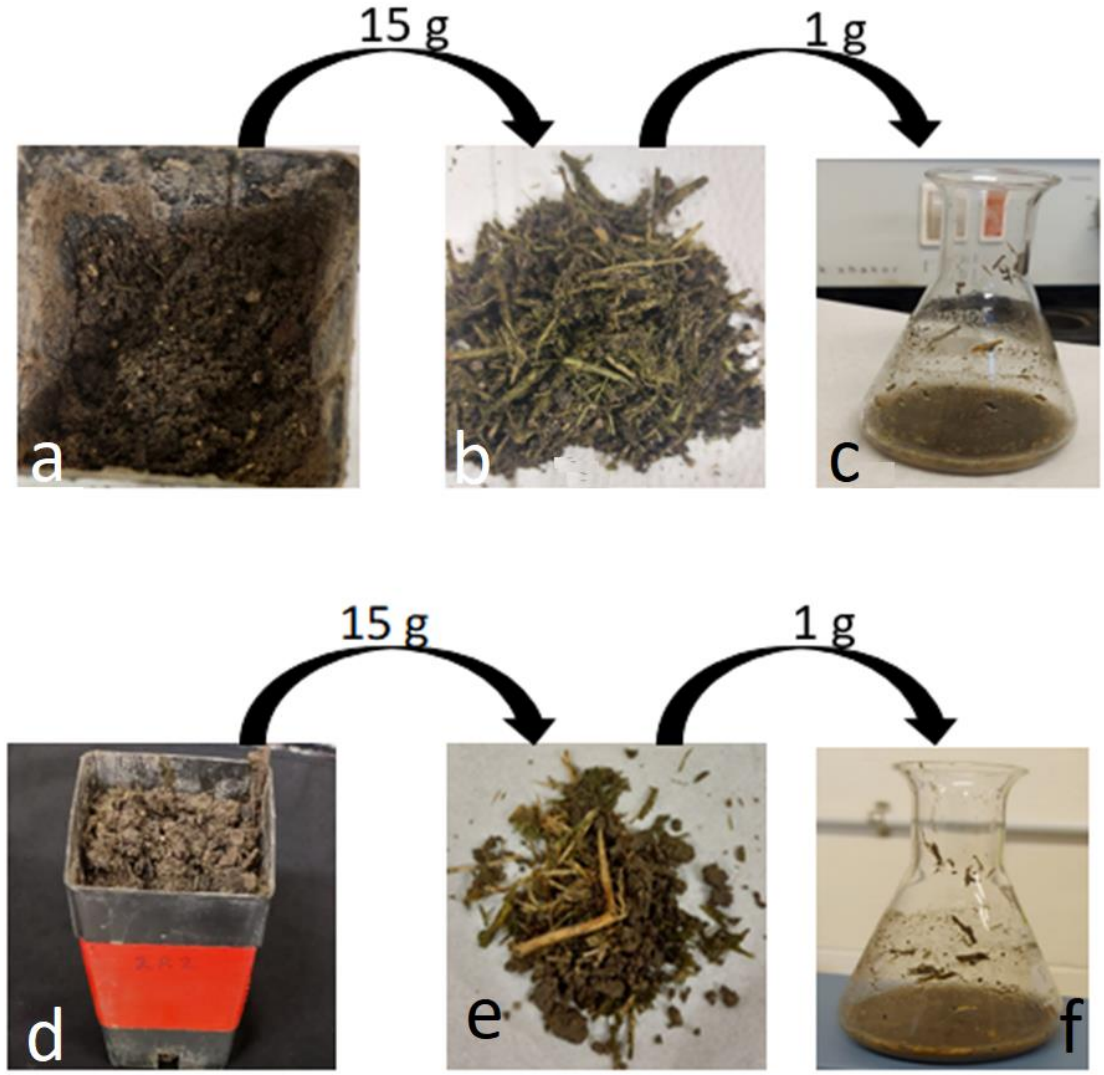

Figure 4. 4 Recovery of bean plant material from pots. a) Partially decomposed buried bean material after removal of the 5 cm layer of the potting mix: soil mixture; b) recovered bean material and associated soil on tissue paper; c) sample (1 g) diluted in sterile distilled water; d) partially decomposed surface bean material in the pot; e) recovered surface bean material and associated soil on tissue paper; f) sample (1 g) diluted in sterile distilled water.

4.2.8 Confirmation of the identity of recovered isolates as *Colletotrichum* species

After the colonies morphologically identified as *Colletotrichum* species were counted on each of the 30 plates for each of the treatment, 15 plates (50%) with more than 5 colonies were randomly selected and all the colonies on these plates were numbered and a number randomizer was used to select 5 colonies from each plate, giving a total of 600 colonies. Each of these isolates was sub-cultured onto separate PDA plates and incubated for 48 hr at 25^{o}C under a 12-12 hr photoperiod. The colonies were subsequently sub-cultured to obtain pure cultures as described in Section 2.3 and their colony characteristics compared with the inoculating *C. acutatum* and *C. gloeosporioides* isolates C.a4 and C.g3, respectively. From the 600 sub-cultures, only 160 (26%) of these were able to be isolated into the pure culture because of the contamination with other soil fungi and bacteria. These were then coded as Sc1-Sc160 and morphologically examined in detail for colony colour, growth rate, conidia shape and size, the morphology of conidiophores, appressoria, and perithecia as described in Section 2.2.2.

4.2.8.1 Morphological analysis

Colony colour was determined for isolate colonies grown on PDA in constant light after 5 days using colour charts (Rayner, 1970). Colony growth was determined by measuring the diameter of the colony in two perpendicular directions after 7 and 14 days of incubation at 25^{o}C under continuous near-ultraviolet light. The growth rate between 7 and 14 days was calculated (mm/day). Morphological structures were observed using the slide culture technique after mounting in 0.5 µL lactic acid and lactophenol cotton blue. Perithecia production was observed under a stereo microscope after 14 days' incubation. Conidiophores were assessed from 21-day-old cultures under a compound microscope. Conidia were harvested from 14-day-old cultures and resuspended in sterile water. Conidia lengths and widths (µm) for each isolate were measured at X400 under phase contrast (Du *et al.*, 2005). Conidial morphology was examined in standard condition (Noireung *et al*., 2021). Plates were maintained in a chamber without humidity control at 25°C and held under a fluorescent light/dark cycle of 12/12 hr. The conidia shape and size were determined using a compound microscope at X400 and an Artray Artcam 300MI digital camera system after mounting in lactophenol cotton blue. At least 3 observations were recorded. Appressoria were induced using the slide culture technique as described by Johnston & Jones (1997) and the morphology observed under a compound microscope at X400 as described in Chapter 2, Section 2.2.2. The morphological observations were used to categorise each isolates into different groups.

4.2.8.2 Molecular identification

For each group, the identity of representative isolates (20%) from each morphological group were confirmed by PCR and sequencing of the ITS gene region as described in Sections 3.2.7.2 and 3.2.7.3.

4.2.9 Statistical analysis

All data were analysed using GenStat statistical package (version 12 edition). The mean CFU was compared between treatments and isolate. Treatment interactions were determined at 95% Confidence Interval to ascertain significant difference using analysis of variance (ANOVA) in a completely randomised block design. Fisher's protected least significant difference test was used to ascertain treatments that differed statistically from each other. Descriptive statistics using tables and graphs were used to present morphological data. Bonferroni test was conducted to show where isolate groups differed significantly in terms of colony growth, conidia length, and width.

4.3 Results

The preliminary experiment showed that Prune agar was the most effective media for the recovery of *Colletotrichum* species. Dilution factor 10^4 was selected since it produced less crowded colonies on plates (Figure 4.4 b). No colonies morphologically identified as *Colletotrichum* species were isolated from the potting mix:soil mixture used for the experiment, indicating there was no background inoculum of *Colletotrichum* species in the soil used for the experiment.

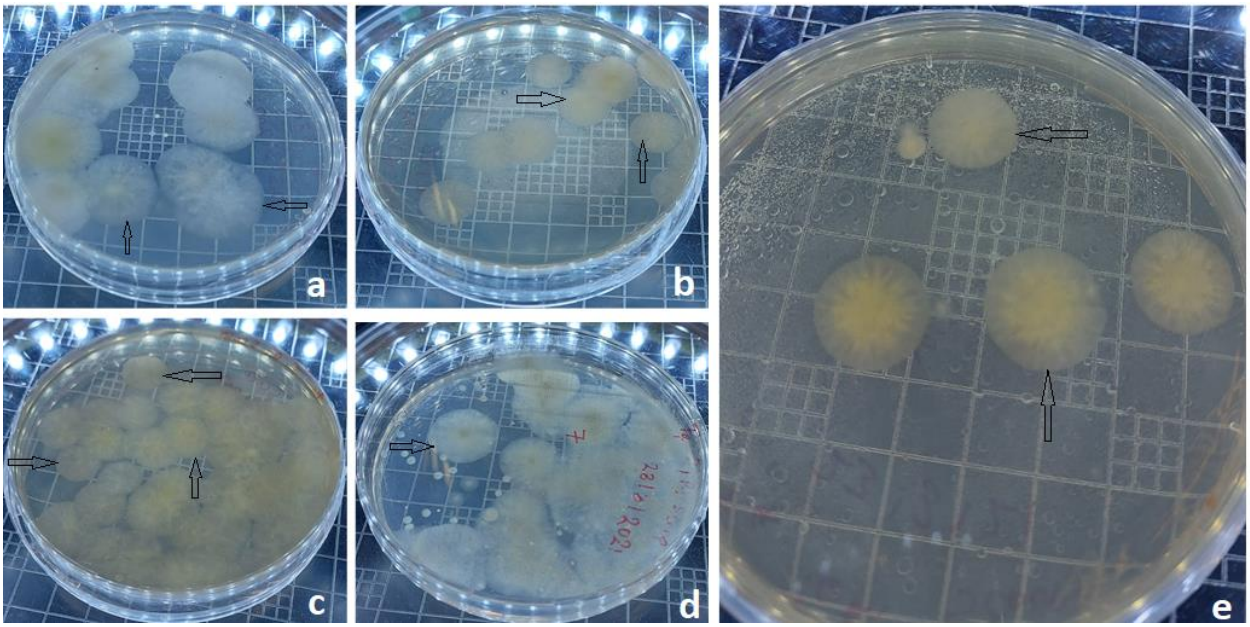

Figure 4. 5 Representative plates for different media used to determine the best media for recovery of *Colletotrichum* colonies (arrows) from soil: potting mix, where the 10^4 dilution factor was plated on the media. a) Water agar, b) prune agar, c) full strength PDA, d) half strength PDA, and e) V8 agar.

After the 6 weeks incubation period, coarse bean tissue material left on the surface had not decomposed. Blended bean tissue material left on the surface and coarse material buried 5 cm deep in the potting mix: soil mixture achieved partial breakdown. Advanced decomposition was recorded only for the buried blended bean material. Colonies morphologically identified as C*olletotrichum* species were recovered from bean tissue harvested from all of the inoculated treatments after the 6 week incubation period.

For *C. acutatum* inoculated materials*,* there was a significant effect of treatment ($P<0.001$; Appendix C, Table C.3) on the recovery of *Colletotrichum* species colonies from the bean plant material, with recovery being significantly higher for all the *C. acutatum* C.a4 inoculated treatments compared with the untreated controls (Fig 4.6 A). The highest recovery was in *C. acutatum* C.a4 inoculated blended and buried bean tissue (1.67×10^5 CFU/g plant tissue) which was significantly higher than all other treatments. This was followed by the inoculated coarsely cut and buried bean tissue (1.36×10^5 CFU/g plant tissue) which was significantly higher than both surface, inoculated blended (6.73×10^4 CFU/g plant tissue) and surface inoculated course (5.43×10^4 CFU/g plant tissue) bean tissue. There was no difference in the recovery of *Colletotrichum* species from the bean tissue among the untreated controls, with low levels of *Colletotrichum* colonies recovered from bean tissue in all control treatments (8.9-8.3 $\times$ 10^3 CFU/g plant tissue) except for blended bean tissue on the surface where no *Colletotrichum* colonies

were recovered. Colonies in the untreated controls took longer to grow, appearing after4 days of incubation compared with after 2 days for the inoculated treatments.

For *C. gloeosporioides* inoculated materials, there was a significant effect of treatment (P-value = < 0.001) (Appendix 3, Table C.4) on the recovery of *Colletotrichum* species colonies from the bean plant's material, with recovery being significantly higher in both the *C. gloeosporioides* C.g3 inoculated blended bean tissue incubated on the surface (9.76×10^5 CFU/g plant tissue) and buried (9.46×10^5 CFU/g plant tissue) than all other treatments (Figure 4.6 B). The frequency of recovery from the *C. gloeosporioides* inoculated course bean tissue buried in the soil differed significantly from all the uninoculated control treatments except the buried uninoculated blended bean tissue. For the inoculated course bean tissue incubated on the surface, recovery of *C. gloeosporioides* did not differ significantly from any of the untreated controls.

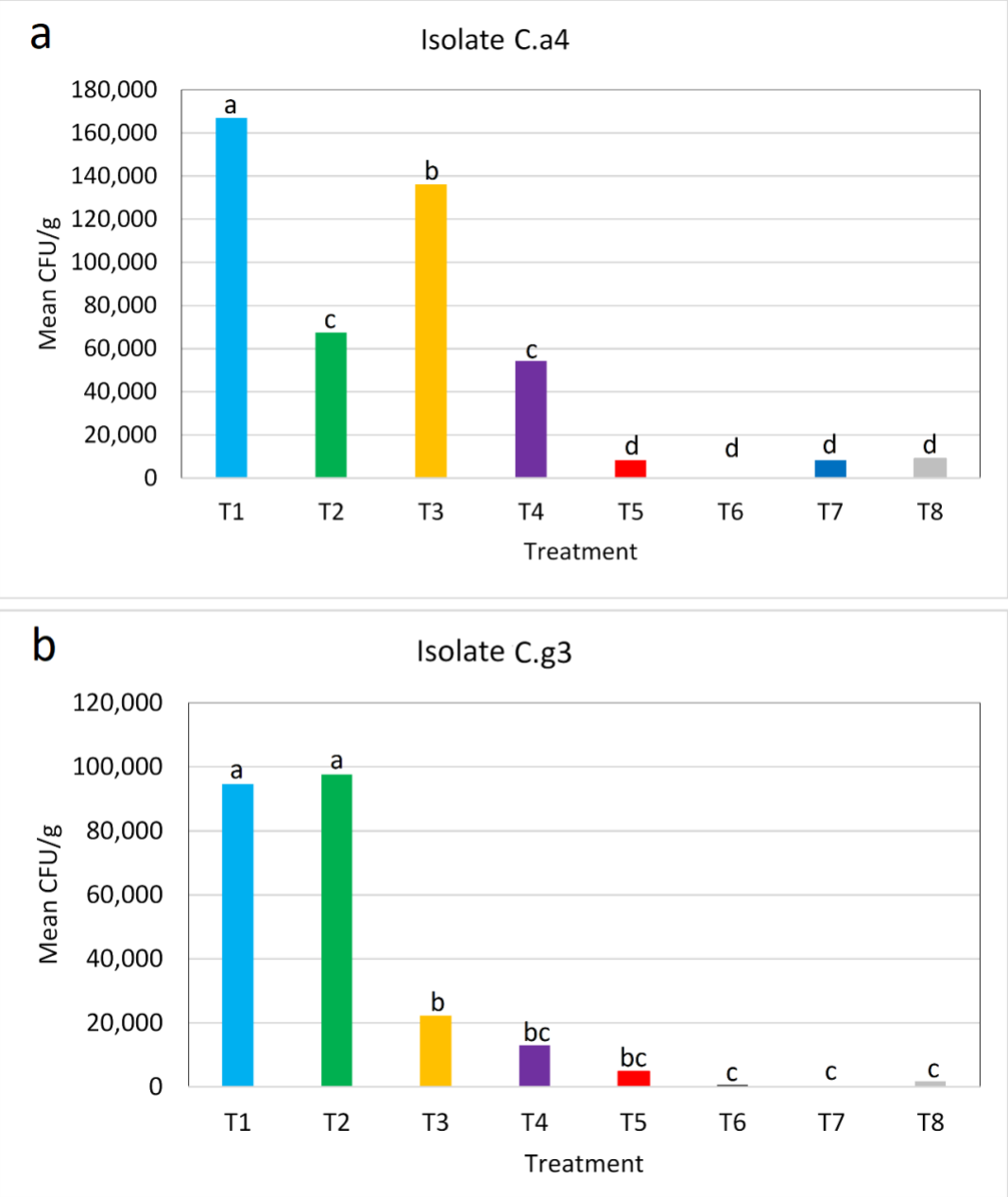

Figure 4. 6 Recovery of colonies (CFU/g crop debris) morphologically identified as *Colletotrichum* species from blended or coarsely chopped *Phaseolus vulgaris* crop debris inoculated with a) *C. acutatum* isolates C.a4 or b) *C. gloeosporioides* isolate C.g3 and buried or left on the surface of the potting mix:soil mixture in pots after 6 weeks incubation in a glasshouse. Treatment 1, buried, inoculated blended bean tissue; Treatment 2, surface, inoculated blended bean tissue; Treatment 3, buried inoculated, coarsely cut bean tissue; Treatment 4, surface, inoculated coarsely cut bean tissue; Treatment 5, buried, uninoculated blended bean tissue; Treatment 6, surface, uninoculated blended bean tissue; Treatment 7, buried uninoculated coarsely cut bean tissue, and Treatment 8, surface, uninoculated coarsely cut bean tissue. Bars with different letters are significantly different based on Fisher's unprotected least significant difference test.

Morphological and cultural characteristics

Of the 600 isolates sub-cultured from the dilution plates, 592 (98.6%) exhibited morphologies similar to *Colletotrichum* species From these, 80 of the isolates for both *C. acutatum* (SC.a) and *C. gloeosporioides* (SC.g) were randomly selected (a total of 160 isolates; 26%) and were further analysed morphologically. The 160 isolates were classified into four morphological groups based on their morphological and cultural characteristics. Group 1 included 67 isolates that matched the description of the *C. gloeosporioides* species complex; Group 2 comprised of 13 isolates that were consistent with the description of *C. fructicola*; Group 3 consisted of 64 isolates that had the characteristics of *C. acutatum* complex, and Group 4 had 7 isolates which exhibited characteristics similar to *C. truncatum*. All isolates in Groups 1 and 2 originated from *C. gloeosporioides*, C.g3 treatments (including the relevant controls), whilst all isolates in Groups 3 and 4 were recovered from *C. acutatum*, C.a4 treatments (including the relevant controls). Isolates morphologically identified as *C. gloeosporioides* (Group 1) and *C. acutatum* (Group 3) were the most predominant groups, consisting of 41.8% and 40.0% of the total isolates, respectively. A summary of the morphological data for the *Colletotrichum* species in Groups 1-4 are presented in Table 4.1.

The colony colour of each isolate group was observed on PDA after 5 days. Group 1 isolates produced pale mouse grey colonies, with sparse white aerial mycelia on both the front and reverse sides of the plate (Figure 4.7A and B). Group 2 showed pale purplish-grey colonies on PDA (Figure 4.7 A and B). Group 3 isolates presented as pale violet colonies on the upper side with the production of dense smoke grey aerial mycelia near the inoculum point. The reverse side had a lavender grey colony colour (Figure 4.7 A and B). The colony colour of Group 4 isolates was pinkish on the upper side (Figure 4.7 A). The reverse side of the colonies was pale grey in colour (Figure 4.7 B). Orange coloured spore masses were produced on all colonies after 21 days of growth (Figure 4.7 D).

There was a significant difference ($P = < 0.001$) (Appendix C, Table C.5) in the mean growth rate between the different Groups. Group 1 isolates had a significantly higher growth rate compared (6.6 ± 1.6) with the other three groups. Isolates from Group 4 achieved a significantly lower growth rate (4.5 ± 1.2 mm/day) (Table 4.1).

The conidia varied in shape between the different groups, with Group 1 and 2 isolates having cylindrical conidia with rounded ends, Group 3 fusiform conidia, and Group 4 falcate conidia with gradual tapering towards each end. The shape of conidia produced by isolates in Groups 3 and 4 was different from those produced by isolates in Groups 1 and 2 in terms of shape, allowing these groups to be easily distinguished from one another (Table 4.1).

The mean length of conidia between isolates in the different groups, varied significantly (P-value = 0.001) (Appendix C, Table C.6) with the conidial length of Group 4 isolates being longer (24.1 ± 1.1) and Group 3 shorter (14.0 ± 2.0) than other groups. Similarly, the width of Group 4 was significantly narrower (P=0.001; Appendix C, Table C.7) (3.7 ± 0.3) than all others (Table 4.1).

There was no difference in the morphological characteristics of the appressoria of isolates across the different groups, with these varying from being ovoid to slightly irregular in shape and light brown to brown in colour. Perithecia were produced in 20-day-old colonies as black hard, dark-brown, or black structures. These were either distributed irregularly within the colony or formed aggregates in bands or rows where they met (Figure 4.7 G). The conidiomata of isolates in all the groups were hyaline to pale brown, simple or septate, rarely branched, and smooth-walled. Two types of conidiophores were observed: isolates in Groups 1 and 2 produced nearly cylindrical, but narrower conidiophores towards the end whilst isolates in Groups 3 and 4 produced conidiophores that were cylindrical, with a truncate top.

Table 4. 1 Summary of the colony, conidia, and appressoria morphological characteristics used to group the isolates into the four groups representing the different species. Mean mycelial growth rate (mm/day± SE; range), conidial length and width (µm± SE; range), and shape. Means in each column followed by different letters are significantly different based on Duncan's multiple range test at P=0.05

Group (no of isolates)	**Species**	**Colonies appearance**	**Growth rate (mm/day)**	**Conidia**			**Appressoria characteristics**
				Length (µm)	Width (µm)	Shape	
Group 1 (67)	*C. gloeosporioides*	pale yellowish colonies, reverse white	6.6 ± 1.6 a 3.3-12.7	16.5 ± 1.7 c* 11.2-19.8	5.5 ± 0.9 b* 3.3-7.3	Cylindrical	Light brown to brown, ovoid
Group 2 (13)	*C. fructicola*	white to black green	5.9 ± 2.0 b* 4.2-12.4	16.2 ± 1.8 c* 12.4-19.4	5.5 ± 1.2 b* 2.5 - 7.7	Cylindrical	Light brown to brown, ovoid, and clavate.
Group 3 (64)	*C. acutatum*	pinkish on both front and reverse	5.8 ± 1.4 b* 4.0-12.5	14.0 ± 2.0 b 9.0 – 18.3	5.2 ± 0.3 b* 3.7 - 5.9	Fusiform conidia shape with sharp ends	Brown, ovate
Group 4 (16)	*C. truncatum*	Pinkish to pale grey on front and reverse	4.5 ± 1.2 c 3.1-8.1	24.1 ± 1.1 a 22.2-27.1	3.7 ± 0.3 d 2.7 - 4.9	Falcate	Brown, ovate, and ellipsoidal.

[t] the numbers shown in parentheses represent the number of isolates in each group. *The mean values within a column with the same letter do not differ significantly according to Duncan's multiple range test at P=0.05.

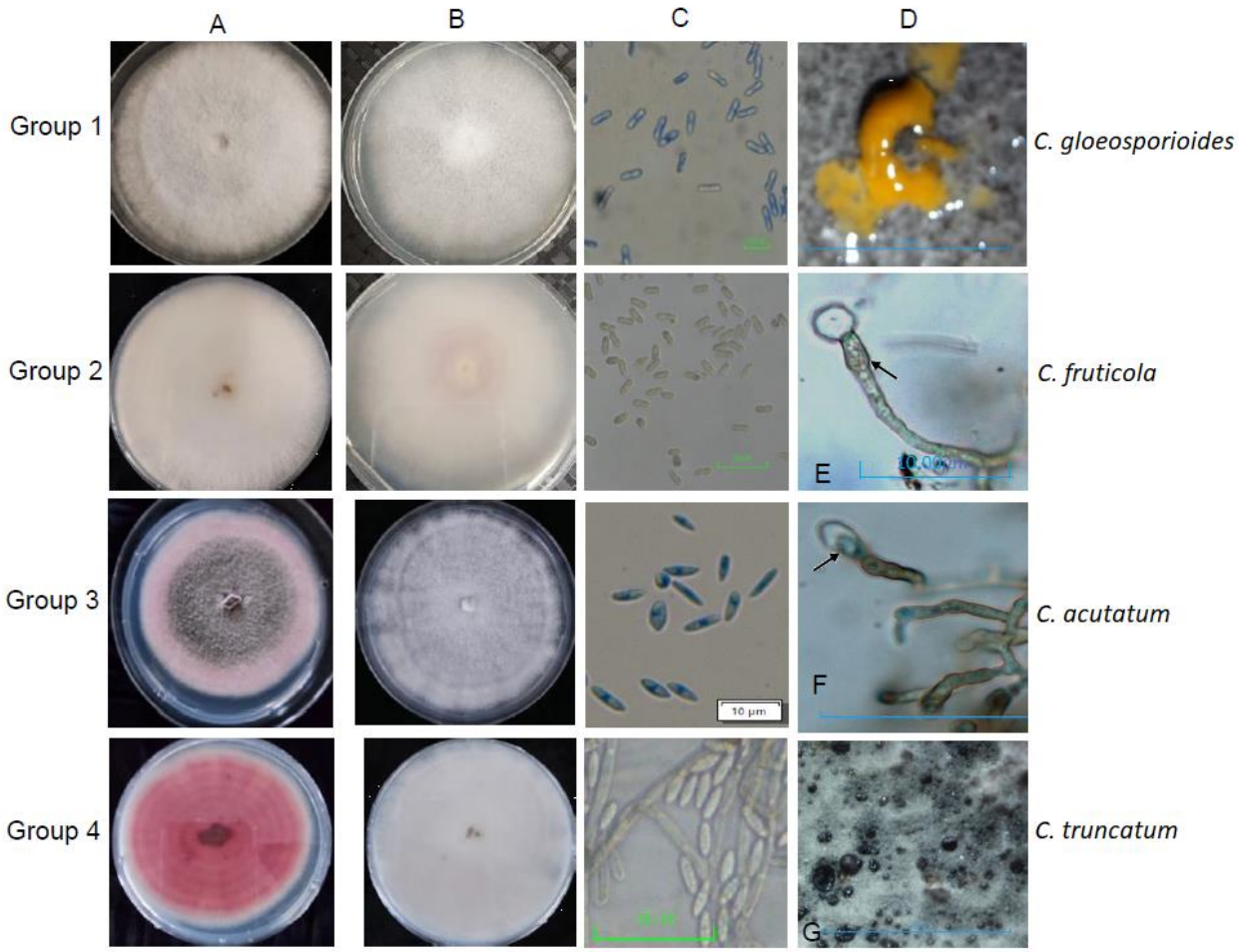

Figure 4. 7 Morphology and cultural characteristics of *Colletotrichum* species recovered from *Phaseolus vulgaris* debris. (A) Upper view of a colony on PDA; (B) reverse view of the colony on PDA; (C) micrographs of conidia of *Colletotrichum* species; (D) orange coloured spore masses produced in culture (conidiomata); (E and F) micrographs of appressoria of *Colletotrichum* species (shown by the arrows); (G) micrographs of perithecia of *Colletotrichum* species Scale bars = 10-20 µm for (C); 10 µm for (D, E, and F); 20 µm for (G).

Molecular identification of species

The expected 560 bp PCR product was amplified for all isolates (Figure 4.8). Of the 13 isolates morphologically identified as *C. gloeosporioides,* the sequences of 8 isolates were 99.4-100% similar to sequences of *C. gloeosporioides* obtained from the NCBI database and therefore confirmed to be *C. gloeosporioides* (Table 4.2) (Appendix C, Figure C.7). The sequences of three isolates Scg2, Scg5, and Scg9) had 89-100% identity with *Colletotrichum* sp. sequences. However, the sequences of the two remaining isolates (Scg1 and Scg4) had 100% identity with *C. kahawae* and *C. fioriniae* sequences. For the three isolates morphologically identified as *C. fruticola* (Scf11, Scf12, and Scf13), the sequence of only one isolate (Scf12) had 100% identity to *C. fructicola*, whereas the sequence of Scf11had 100%

identity to *C. fioriniae* and Scf13 100% identity to *C. kahawae*. For the 13 isolates morphologically identified as *C. acutatum,* the sequences of 9 were 100% identical to sequences of *C. acutatum* obtained from the NCBI database and therefore confirmed to be *C. acutatum* (Table 4.3). The sequences of three isolates Sca11, Sca12, and Sca13) had 100% identity with *Colletotrichum* sp. sequences. For the three isolates morphologically identified as *C. truncatum* one isolate each was confirmed *C. fructicola* (Sct14), *C. lipini* (Sct15), and *C. fioriniae* (Sct16) respectively, with 100% identity. When the representative query isolates (Scg1-Scg10) and (Sca1-Sca13) recovered from infected materials were compared with the sequence of the original isolates used to inoculate (C.g3) and (C.a4) using BLASTN in NCBI, they showed 90.12-100% and 99.15-100% similarity, respectively (Appendix C, Table C.9 and C.10).

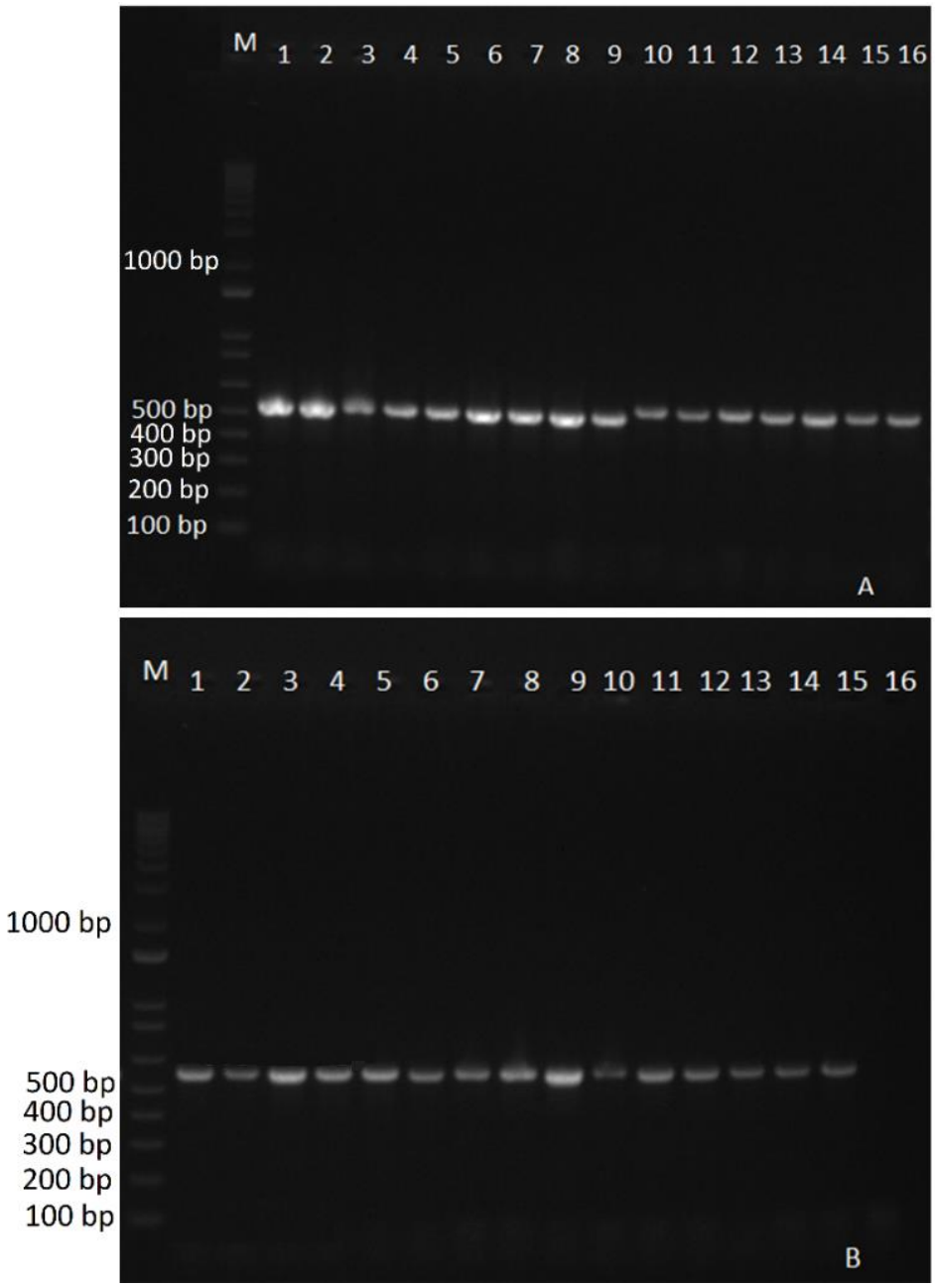

Figure 4. 8 1% agarose gel of PCR products produced by amplifying DNA from isolates of *Colletotrichum* species recovered from inoculated bean tissue buried in the soil or incubated on the surface of the pots using primers ITS1 and ITS4. (A) Colonies recovered from *C. gloeosporioides* treatments (Lanes numbered 1-10 and 14-16 are Scg1 – Scg13, Scg14-Scg16; 11-13 are Scf11 - Scf13; (B) Colonies recovered from *C. acutatum* treatments (Lanes numbered 1-13 are Sca1 – Sca13, 14-16 are Sct14 - Sct16; M = molecular size marker (1 kb + ladder).

Table 4. 2 Sequencing results for the isolates morphologically identified as, *C. gloeosporioides* (Scg1-10, Scg14-16) and *C. fructicola* (Scf11-13).

Isolates	Identity	Query Cover	E- value	% identity	Length	GenBank Accession No.
Scg1	*Colletotrichum kahawae*	100%	0	100	588	MT133276.1
Scg2	*Colletotrichum gloeosporioides*	100%	0	100	593	MT083978.1
Scg3	*Colletotrichum* sp.	100%	0	100	591	MN944922.1
Scg4	*Colletotrichum fioriniae*	100%	0	98.2	609	MN842794.1
	Colletotrichum acutatum	100%	0	99.63	587	
Scg5	*Colletotrichum* sp.	89%	0	89.75	591	MN944922.1
Scg6	*Colletotrichum gloeosporioides*	100%	0	99.44	557	FJ539189.1
SCg7	*Colletotrichum gloeosporioides*	100%	0	100	593	MT083978.1
SCg8	*Colletotrichum gloeosporioides*	100%	0	100	599	MF495411.1
Scg9	*Colletotrichum* sp.	100%	0	100	593	KT282714.1
Scg10	*Colletotrichum gloeosporioides*	100%	0	100	599	MF495411.1
	Colletotrichum kahawae	100%	0	100	577	MN744289.1
Scf11	*Colletotrichum gloeosporioides*	100%	0	99.82	599	MF495411.1
	Colletotrichum kahawae	100%	0	99.82	577	MN744289.1
Scf12	*Colletotrichum fructicola*	100%	0	100	594	MN871666.1
	Colletotrichum gloeosporioides	100%	0	100	591	MN856423.1
Scf13	*Colletotrichum kahawae*	100%	0	100	556	MN273080.1
	Colletotrichum gloeosporioides	100%	0	100	546	MT083978.1
	Colletotrichum aotearoa	100%	0	100	593	MN273068.1
Scg14	*Colletotrichum gloeosporioides*	100%	0	100	593	MT083978.1
Scg15	*Colletotrichum gloeosporioides*	100%	0	100	593	MT083978.1
Scg16	*Colletotrichum gloeosporioides*	100%	0	99.8	593	MT083978.1

Table 4. 3 Sequencing results for the isolates morphologically identified as *Colletotrichum acutatum* (Sca1- 13) and *C. truncatum* (Sct14-16)

Isolates	Identity	Query Cover	E-value	% Identity	Length	GenBank Accession No.
Sca1	*Colletotrichum fioriniae*	100%	0	100	553	MT466533.1
	Colletotrichum acutatum	100%	0	100	591	MN856415.1
Sca2	*Colletotrichum acutatum*	100%	0	99.61	591	MN856423.1
	Colletotrichum fioriniae	100%	0	99.61	531	MN886535.1
Sca3	*Colletotrichum acutatum*	100%	0	100	591	MN856423.1
	Colletotrichum fioriniae	100%	0	100	531	MN886535.1
Sca4	*Colletotrichum acutatum*	100%	0	100	587	MN856415.1
Sca5	Colletotrichum acutatum	100%	0	100	591	MN856423.1
	Colletotrichum fioriniae	100%	0	100	531	MN886535.1
Sca6	*Colletotrichum acutatum*	100%	0	100	589	MG554643.1
Sca7	*Colletotrichum acutatum*	100%	0	100	591	MN856423.1
	Colletotrichum fioriniae	100%	0	100	531	MN886535.1
Sca8	*Colletotrichum acutatum*	100%	0	99.82	591	MN856423.1
	Colletotrichum fioriniae	100%	0	99.82	531	MN886535.1
Sca9	*Colletotrichum acutatum*	99%	0	100	586	MG553373.1
Sca10	*Colletotrichum acutatum*	100%	0	99.3	608	MH930414.1
Sca11	*Colletotrichum* sp.	100%	0	100	591	MN944922.1
	Colletotrichum fioriniae	100%	0	100	595	MT133297.1
	Colletotrichum acutatum	100%	0	100	591	MN856423.1
Sca12	*Colletotrichum* sp.	100%	0	99.82	591	MN944922.1
Sca13	*Colletotrichum* sp.	100%	0	100	599	LC435467.1
Sct14	*Colletotrichum fioriniae*	100%	0	100	553	MT466533.1
	Colletotrichum lupini	100%	0	100	599	LC206553.1
	Colletotrichum acutatum	100%	0	100	591	MN856423.1
Sct15	*Colletotrichum fioriniae*	100%	0	100	553	MT466533.1
	Colletotrichum acutatum	100%	0	100	591	MN856423.1
Sct16	*Colletotrichum fioriniae*	100%	0	99.82	609	MT133290.1
	Colletotrichum acutatum	100%	0	99.63	587	MN856415.1

4.4 Discussion

The survival of *Colletotrichum acutatum* and *C. gloeosporioides* on finely blended or coarsely chopped debris of *P. vulgaris* left on the soil surface or buried at a depth of 5 cm in the potting mix:soil was investigated in a glasshouse experiment. After six weeks of incubation viable colonies of both *C. acutatum* and *C. gloeosporioides* were recovered from both the inoculated debris left on the potting mix: soil surface or buried at a depth of 5 cm. Overall the recovery was higher for the plant material buried in the potting mix:soil than for the material left on the surface, especially for the blended bean plant material. This could be related to differences in the moisture level of the tissue on the surface compared with that buried in the potting mix: soil mixture used.

Most pathogenic soil fungi grow best at a soil moisture level of 60% WHC (Borowik & Wyszkowska, 2016). Since the material left on the surface was exposed to air drying, the daily watering to attempt to maintain the moisture level at 60% may not have been sufficient to induce conidial germination of the *Colletotrichum* species inoculum and subsequent growth and colonisation of the plant tissues. Freeman *et al.* (2002) reported the viability of conidia of *C. acutatum* and *C. gloeosporioides* to decline rapidly in soils at 22% soil moisture (field capacity), with a 95% reduction in population within 4 to 10 days of exposure. These results agree with that finding and suggest that exposing pathogen-infested plant residues to drying on the soil surface might play a role in reducing the inoculum load for new infections in the next season.

However, it is worth noting that in the present study rather than naturally infected material, plant materials were artificially sprayed with conidial suspensions and then used immediately in the experiment. Thus the results of this study may not reflect what would be expected if naturally infected material was used, since the pathogens are likely to produce perithecia, especially on infected crop material at the end of the season, enabling the pathogens to survive adverse environmental conditions such as drying (de Silvia *et al*., 2017). Further, since the plant debris did not degrade on the soil surface compared with when incorporated into the soil, it is likely that for naturally infected plant materials the pathogens would remain viable to act as a source of inoculum to infect other crops.

The overall increase in recovery of the pathogen from the buried tissue may have also been due to conidia which had not germinated and colonised the tissue and are therefore likely to be washed off the debris into the surrounding potting mix:soil. Therefore, the sampling of the buried material with the

associated potting mix:soil may have influenced the higher recovery of the pathogens in buried tissue which was not the case for the surface material which was easily separated from the potting mix:soil. Future research should attempt to determine the survival of *Colletotrichum* species using naturally infected bean material. Further, to ensure that only the crop debris is sampled, it would be recommended to place the crop debris in mesh bags prior to burial which would facilitate the recovery of the partially decomposed tissue and limit the contamination with the adjacent potting mix:soil.

Higher recovery of both *C. acutatum* and *C. gloeosporioides* from the blended plant material buried in the potting mix:soil, and on the surface for *C. gloeosporioides* compared with coarsely cut tissues was observed. The relative consistency of the *P. vulgaris* material used is likely to have affected the recovery of the pathogens and is probably a result of the increased moisture level in the blended material compared with the coarsely cut material, which may have facilitated the germination and growth of conidia on the material. From these present results, there was a difference in the recovery between the two *Colletotrichum,* species with recovery being higher from plant material inoculated with *C. acutatum* isolate C.a4 (5.4 – 16.7 x 10^4 CFU/g of plant tissue) than from plant material inoculated with *C. gloeosporioides* isolate C.g3 (1.3 – 9.7 x 10^4 CFU/g of plant tissue).

Further, in comparison with the original concentration on the inoculated tissue at the start of the experiment (equivalent to 1.33 x 10^4 conidia/g of plant tissue), there was an increase in the recovery after the 6 weeks incubation period. The increase in CFU was mostly seen in the buried material compared with the material incubated on the potting mix:soil surface indicating that there was an increase in inoculum for both pathogens during the incubation period. This indicates that the conidia had germinated and produced colonies which contributed to the observed increase in CFUs. The blending of the bean material may have contributed to this increase in inoculum due to the release of nutrients from the macerated tissue facilitating germination and subsequent growth and inoculum production by the pathogens.

The survival of *C. acutatum* in plant residues and soil has been previously documented. Parikka and Lemmetty (2010) reported that in Norway, *C. acutatum* was able to survive on artificially infected strawberry plant material debris for at least one winter period when these were dried and then buried in soil or left on the surface of soil. *Colletotrichum acutatum* was also shown to infect several weed species that grew in the trial site further showing the ability of the pathogen to be able to survive both on plant residues and on alternative living host plants, including weeds, for up to one year. Similar

results were reported by Norman and Strandberg (1997) where viable conidia of *C. acutatum* were recovered up to 12 months after incorporation into the soil, and shown to survive on infected leatherleaf fern (*Rumohra adiantiformis*) leaf debris buried in soil under field conditions for up to 105 days, but recovery declined rapidly from 14 days onwards. These reports support our finding on the ability of *C. acutatum* to survive on the debris of several plants when left on the surface or buried in the soil for more than six weeks.

As shown in the present study, *C. gloeosporioides* is also reported to survive in plant debris. Kumari *et al.* (2017) disclosed that *C. gloeosporioides* is able to survive for more than seven months in soil as conidia or mycelium in naturally infected mango debris under adverse conditions. However, survival of the pathogen was shown to reduce with an increase in soil depth and duration of burial of the infected plant residue, thus demonstrating its long-term survival only on the soil surface. In addition, Takushi (2015) found conidial masses of *C. gloeosporioides* to survive on mango leaf debris for more than 10 months under favourable field conditions. These findings are at variance with our results probably because of the differences in the type of infected material used in the studies.

As stated earlier, in the present study, plant material was artificially inoculated by spraying with a conidial suspension of the two pathogens and used immediately to set up the experiment. Although there was an indication from the recovery data that the pathogen conidia germinated and grew on the plant material, it is also likely that colonisation of the tissue might be different to that seen with natural infection whereby the pathogens would be protected within the infected plant tissue (either as mycelium or perithecia) as stated by Rodríguez-Guerra *et al.* (2005). Further, exposure of germinating conidia on the inoculated plant material placed on the potting mix:soil surface under hot and dry greenhouse conditions could have caused their desiccation and loss of viability hence leading to a reduction in the recovery of inoculum from these treatments.

Although the viability of *C. acutatum* and *C. gloeosporioides* on infected crop debris in soil has been shown to decrease over time and depth of burial, their long term survival on infected plant residues as shown in the current study and other studies shows these as potential inoculum reservoirs of new infection to other susceptible hosts. Although this present study was conducted in a glasshouse environment, results from studies carried out under field conditions confirm our findings. Since both *C. acutatum* and *C. gloeosporioides* were also shown to infect common bean (Chapter 3), the ability of the common bean to be an alternative host for these pathogens that cause anthracnose disease of mango

lay a foundation for understanding the epidemiology of anthracnose disease to establish control strategies in a mango cropping system.

After six weeks, there was limited decomposition of the coarse and blended plant material left on the potting mix:soil surface, together with coarse material buried at a depth of 5 cm deep which was likely due to the difference in the texture of the material used (course vs blended), the short duration of the experiment as well as the difference in the moisture content of the material used. The effect of incubation time on the decomposition of the plant materials was evident as after six weeks the lignified parts of the bean plant material had still not decomposed. Ripoche *et al.* (2008) found soil moisture and residue quality as key factors that influenced the rate of decomposition in the field and this agrees with the observations in this study. Plant parts such as stems and the leaf midrib usually remain in the soil for more than five months due to the slow decomposition rate of these recalcitrant organic fractions. Ekefan *et al.* (2000) noted that the survival of *C. gloeosporioides* on crop residues is limited by competition from other soil decomposing microorganisms. Thus this delay in the decomposition of more intact crop residue material may result in increased survival of the pathogens in the field to continue acting as potential reservoirs of inoculum for new infection.

Based on the observed morphological characteristics, 84% of isolates recovered from the *C. gloeosporioides* treatments were identified as *C. gloeosporioides,* and 80% of the isolates recovered from the *C. acutatum* treatments as *C. acutatum*. The remaining isolates exhibited cultural characteristics different from the two isolates used to inoculate the material. Of these, 16% of the isolates recovered from the *C. gloeosporioides* treatments were identified as *C. fructicola,* and 20% of the isolates recovered from the *C. acutatum* treatments as *C. truncatum.* However, sequencing of the ITS region confirmed all the isolates recovered from the *C. acutatum* treatments to be members of the *C. acutatum* species complex and all but one of the isolates recovered from the *C. gloeosporioides* treatments to be members of the *C. gloeosporioides* species complex. As stated by Weir *et al.* (2012c). *Colletotrichum* species identification based on morphological characteristics is very difficult owing to the overlapping features between closely related species. As discussed previously, for accurate species identification, future work should incorporate sequencing of additional gene regions such as partial Actin (ACT), β-tubulin (TUB2), Calmodulin (CAL), Glutamine synthetase (GS), and Glyceraldehyde 3-phosphate dehydrogenase (GPDH) (Cai *et al.*, 2009; Doyle *et al.*, 2013a; Weir *et al.*, 2012c).

The results of our study showed that prune agar was the most suitable media to recover *Colletotrichum* species from plant debris incorporated into the potting mix:soil mixture. This could be due to the acidity of the medium, which partially suppressed the growth of other microorganisms in the potting mix:soil media used for the experiment.

The recovery of *Colletotrichum* colonies in some control treatment pots could be due to cross-contamination of material during the experimental setup since no *Colletotrichum* colonies were recovered in the initial testing of the potting mix: soil prior to setting up the experiment. Cross-contamination of the bean crop material in the controls due to splash dispersal during watering of the pots was unlikely since care was taken to minimize any splash by watering using a syringe. However, fungal spores have been shown to be transported by soil insects such as mites and collembolans (Dromph & Borgen, 2001; Williams *et al.*, 1998) and this may have contributed to the transfer of conidia from the inoculated material to the adjacent untreated control pots.

4.4.1 Conclusions

The findings of the study reveal the potential of *C. acutatum* and *C. gloeosporioides* to survive on common bean plant debris both when buried in soil and or incubated on the soil surface for six weeks. Strict phytosanitary measures, including removal of previous crop debris in a mango cropping system such as in Uganda where the common bean is the main alternate crop should be enforced. This will ensure the removal of potential sources of inoculum available to initiate new infections. Future research should investigate the survival of *C. acutatum* and *C. gloeosporioides* on naturally infected bean material at different soil depths under varying field conditions.

Chapter 6. Concluding Discussion

Colletotrichum species are economically important pathogens that infect a wide range of hosts leading to substantial production losses in the tropical and sub-tropical regions of the world. The fungus causes anthracnose disease of cereals, legumes, vegetables, perennial crops, and tree fruits. The susceptibility of a wide range of host plants to *Colletotrichum* species makes control efforts very difficult. Most importantly, the infection of alternate crop species by these pathogens presents a potential primary inoculum source for a new infection to other susceptible hosts in mixed cropping systems. Fungal disease control in most production systems has often relied on chemical control methods. Chemical controls can be sustainable when used properly. However, it's less economical in some cropping systems like those in Uganda. Thus, developing disease control strategies that are cost-effective is required. Understanding the epidemiology of the pathogen on the different host species is needed to inform disease control programs or interventions in a mixed cropping system such as that widely used in Uganda. Elimination of sources of inoculum is an important element to consider when planning for effective disease control in the field.

This present study aimed to characterize the identity of *Colletotrichum* species isolates obtained from naturally infected plant materials *in vitro* based on colony growth and morphology under different light and temperature regimes and sequencing of the ITS gene region, to test the pathogenicity of isolates of *C. acutatum* and *C. gloeosporioides* on *Phaseolus vulgaris* under glasshouse conditions, and to assess the survival of *C. acutatum* and *C. gloeosporioides* in the debris of *P. vulgaris* when on the soil surface or buried.

Colletotrichum species identification using morphological characteristics provided the basis for identification but was limited to distinguishing between species due to overlapping of features between cryptic species complexes. Similarly, sequencing of the ITS gene region identified the isolates as members of *C. acutatum* and *C. gloeosporioides* species complexes and was insufficient to delineate between species within these complexes. These results show that for precise identification of *Colletotrichum* species a combined approach, employing both morphological and molecular methods such as the sequencing of multigene regions or multilocus phylogenic analysis is required.

Colony growth and conidial production of *C. acutatum* and *C. gloeosporioides* species complex isolates were higher under 12 hr light: 12 hr dark incubation than under constant dark incubation. Incubation

temperature affected colony growth and conidial production of all isolates with the optimum temperature for growth and sporulation of most isolates of *C. acutatum* and *C. gloeosporioides* being 25 – 30^{o}C. Growth and sporulation of all isolates were lowest at 15^{o}C. These results indicate that exposing inoculum sources to extreme temperatures and light regimes could reduce the chances of and/ or severity of infection caused by *Colletotrichum* species in any production system. Future work assessing the effect of a wider temperature range (5-40^{o}C) on growth and sporulation of *Colletotrichum* species under field conditions such as varying soil temperatures is desired.

In the pathogenicity testing, isolates of both *C. acutatum* and *C. gloeosporioides* were infective on the green bean, *Phaseolus vulgaris,* under glasshouse conditions with there being no difference in incidence or disease severity between the inoculum concentrations tested. This result demonstrates the ability of *C. acutatum* and *C. gloeosporioides* to infect multiple hosts. Since *C. acutatum* and *C. gloeosporioides* are known to be responsible for most anthracnose diseases in fruit trees, including mango, and have been shown in this project to be able to infect *Phaseolus vulgaris,* avoiding planting beans together with other susceptible hosts such as mango is recommended. This would reduce primary inoculum sources for new infection in these cropping systems. In addition, any disease control strategy should take into consideration all of the susceptible crops grown to avoid spread of these diseases from one host to another especially in a mixed cropping system. Further pathogenicity studies on *P. vulgaris* using isolates from naturally infected mango fruit should be conducted under field conditions including determining at which growth stage the bean plant is most susceptible to these pathogens. The experiments should also include determining the susceptibility of different *P. vulgaris* cultivars, including those commonly grown in mixed cropping systems in Uganda.

To determine the ability of *C. acutatum* and *C. gloeosporioides* to survive on inoculated *P. vulgaris* plant tissue, the pathogens were recovered from artificially inoculated material left on the surface of the potting mix:soil and buried at a depth of 5 cm after six weeks. Both *C. acutatum* and *C. gloeosporioides* were shown to be recovered from all the crop material after six weeks indicating the potential for infected bean material to act as an inoculum source. Recovery of both *C. acutatum* and *C. gloeosporioides* was higher from inoculated blended and buried crop material than coarsely cut materials. The results of the study indicated that the survival of these fungal pathogens, *Colletotrichum* species on crop debris is affected by the moisture level of both the material and the soil. Thus, incorporating strict phytosanitary measures in the design of anthracnose disease control strategies is

pertinent. Measures such as the removal of crop debris immediately after harvest are recommended to eliminate potential sources of inoculum for new infections in the field.

In light of our present findings, subsequent studies utilizing naturally infected plant materials left on the surface or buried in the field under different soil depths and types are suggested. Alternatively, future studies under local conditions in Uganda should utilise pre-infected leaf and stem material (instead of spray inoculated debris) and to contain the material in open mesh (e.g window mesh) bags for easy retrieval. Further, an evaluation of the effect of cropping season on the survival of the pathogens on plant debris should also be carried out.

References

Acema, D., Asiku, B., Odama, E., & Egama, D. (2016). Assessment of Mango Pests, Diseases and Orchard Management Practices in West Nile Zone of Uganda. *Agriculture, Forestry and Fisheries*, *5*(3), 57-63.

Adaskaveg, J., & Hartin, R. (1997). Characterization of *Colletotrichum acutatum* Isolates Causing Anthracnose of Almond and Peach in California. *Phytopathology*, *87*(9), 979-987.

Agrios, G. (2004). Plant Pathology [Electronic Resource]. In: Elsevier, EBL: Burlington.

Allen, D., & Edje, O. (1990). Common Bean in African Farming Systems. Progress in improvement of common bean in Eastern and Southern Africa. Network on Bean Research in Africa Workshop Series,

Alves, S., & Moraes, S. (1998). Quantificação De Inóculo De Patógenos De Insetos. *Controle microbiano de insetos*, *2*, 765-778.

Arauz, L. F. (2000). Mango Anthracnose: Economic Impact and Current Options for Integrated Managaement. *Plant disease*, *84*(6), 600-611.

Arauz, L. F., Wang, A., Duran, J. A., & Monterrey, M. (1994). Causas De Perdmas Poscosecha De Mango a Nlvel Mayorista En Costa Rica I. Agronomfa Costarricense *18(1)*47-51.

Archana, S., Raguchander, T., & Prabakar, K. (2018). Detection of-Tubulin Gene from Benomyl Sensitive Isolates of *Colletotrichum gloeosporioides* Causing Anthracnose Disease in Mango. *African Journal of Microbiology Research*, *12*(33), 806-814.

Auyong, A. S. M., Ford, R., & Taylor, P. W. J. (2012). Genetic Transformation of *Colletotrichum truncatum* Associated with Anthracnose Disease of Chili by Random Insertional Mutagenesis. *Journal of basic Microbiology*, *52*(4), 372-382.

Awori E, K. M., Souza TLPO, Vieira AF, Nkalubo ST, et al. (2018). Resistance Sources to Bean Anthracnose Disease in Uganda and Brazil. . *J Agri Sci Food Res*, *9: 225*.

Barcelos, Q. L., Pinto, J. M., Vaillancourt, L. J., & Souza, E. A. (2014a). Characterization of *Glomerella* Strains Recovered from Anthracnose Lesions on Common Bean Plants in Brazil. *PLOS ONE*, *9*(3), e90910. https://doi.org/10.1371/journal.pone.0090910

Barcelos, Q. L., Pinto, J. M. A., Vaillancourt, L. J., & Souza, E. A. (2014b). Characterization of *Glomerella* Strains Recovered from Anthracnose Lesions on Common Bean Plants in Brazil. *PLOS ONE*, *9*(3), e90910. https://doi.org/10.1371/journal.pone.0090910

Barimani, M., Pethybridge, S., Vaghefi, N., Hay, F., & Taylor, P. (2013). A New Anthracnose Disease of Pyrethrum Caused by *Colletotrichum tanaceti* Sp. Nov. *Plant Pathology*, *62*(6), 1248-1257.

Barquero Quirós, M., Peres, N. A., & Arauz, L. F. (2013). Presence of *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* on Leatherleaf Fern, Key Lime, Papaya, Star Fruit and Mango in Costa Rica and Florida (United States). *Agronomía Costarricense*, *37*(1), 23-38.

Bellucci, E., Bitocchi, E., Rau, D., Rodriguez, M., Biagetti, E., Giardini, A., . . . Papa, R. (2014). Genomics of Origin, Domestication and Evolution of Phaseolus Vulgaris. *Genomics of plant genetic resources*, 483-507.

Bertetti, D., Gullino, M. L., & Garibaldi, A. (2009). Effect of Leaf Wetness Duration, Temperature and Inoculum Concentration on Infection of Evergreen Azalea by *Colletotrichum acutatum*, the Causal Agent of Anthracnose. *Journal of Plant Pathology*, *91*(3), 763-766. http://www.jstor.org/stable/41998702

Beshir, H. M., Walley, F. L., Bueckert, R., & Tar'an, B. (2015). Response of Snap Bean Cultivars to Rhizobium Inoculation under Dryland Agriculture in Ethiopia. *Agronomy*, *5*(3), 291-308.

Bhagya, N., Sheik, S., Sharma, M. S., & Chandrashekar, K. (2011). Isolation of Endophytic *Colletotrichum gloeosporioides* Penz. From Salacia Chinensis and Its Antifungal Sensitivity. *Journal of Phytology*, *3*(6).

Bitocchi, E., Bellucci, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E., . . . Logozzo, G. (2013). Molecular Analysis of the Parallel Domestication of the Common Bean (*Phaseolus vulgaris*) in Mesoamerica and the Andes. *New Phytologist*, *197*(1), 300-313.

Bitocchi, E., Nanni, L., Bellucci, E., Rossi, M., Giardini, A., Zeuli, P. S., . . . Attene, G. (2012). Mesoamerican Origin of the Common Bean (*Phaseolus Vulgaris L.*) Is Revealed by Sequence Data. *Proceedings of the National Academy of Sciences*, *109*(14), E788-E796.

Borowik, A., & Wyszkowska, J. (2016). Soil Moisture as a Factor Affecting the Microbiological and Biochemical Activity of Soil. *Plant, Soil and Environment*, *62*(6), 250-255.

Broughton, W. J., Hernández, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (*Phaseolus* Spp.) – Model Food Legumes. *Plant and Soil*, *252*(1), 55-128. https://doi.org/10.1023/A:1024146710611

Brown, A., Sreenivasaprasad, S., & Timmer, L. (1996). Molecular Characterization of Slow-Growing Orange and Key Lime Anthracnose Strains of *Colletotrichum* from Citrus as *C. acutatum*. *Phytopathology*, *86*(5), 523-527.

Buruchara, R. (2007). Background Information on Common Beans (*Phaseolus vulgaris L.)*. In Biotechnology, Breeding & Seed Systems for African Crops. The Rockefeller Foundation, Nairobi.

Busby, P. E., Ridout, M., & Newcombe, G. (2016). Fungal Endophytes: Modifiers of Plant Disease. *Plant molecular biology*, *90*(6), 645-655.

CABI. (2020). *Colletotrichum acutatum (Black Spot of Strawberry)* Retrieved July 30 from https://www.cabi.org/isc/datasheet/14889#tosymptomsOrSigns

Cai, L., Hyde, K., Taylor, P., Weir, B., Waller, J., Abang, M., . . . Liu, Z. (2009). A Polyphasic Approach for Studying *Colletotrichum*. *Fungal Diversity*, *39*(1), 183-204.

Calo, L., García, I., Gotor, C., & Romero, L. C. (2006). Leaf Hairs Influence Phytopathogenic Fungus Infection and Confer an Increased Resistance When Expressing a *Trichoderma* A-1, 3-Glucanase. *Journal of Experimental Botany*, *57*(14), 3911-3920.

Cannon, P., Damm, U., Johnston, P., & Weir, B. (2012). C*olletotrichum*–Current Status and Future Directions. *Studies in mycology*, *73*, 181-213.

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T., & Toker, C. (2018a). The Nutritional Content of Common Bean (*Phaseolus vulgaris L.*) Landraces in Comparison to Modern Varieties. *Agronomy*, *8*(9), 166. https://doi.org/10.3390/agronomy8090166

CGIAR. (2015). Common Bean. Retrieved on Dec. 10, 2015 from 10, 2015 from Http://Www.Cgiar.Org/Our-Research/Cropfactsheets/Beans/.

Chakraborty, N., Mukherjee, K., Sarkar, A., & Acharya, K. (2019a). Interaction between Bean and *Colletotrichum gloeosporioides*: Understanding through a Biochemical Approach. *Plants (Basel, Switzerland)*, *8*(9), 345. https://doi.org/10.3390/plants8090345

Chakraborty, S., & Datta, S. (2003). How Will Plant Pathogens Adapt to Host Plant Resistance at Elevated Co2 under a Changing Climate? *New Phytologist*, *159*(3), 733-742.

Chang, J., Boer, R., Crous, P., & Taylor, P. (2014). Biology of Foliar and Root Infection of Potato Plants by *Colletotrichum coccodes* in Australia. Phytopathology, 104(11), 24-24.

Ciampi-Guillardi, M., Baldauf, C., Souza, A. P., Silva-Junior, G. J., & Amorim, L. (2014). Recent Introduction and Recombination in *Colletotrichum acutatum* Populations Associated with Citrus Postbloom Fruit Drop Epidemics in São Paulo, Brazil. *Phytopathology*, *104*(7), 769-778.

Crouch, A., White, Hillman, Bradley (2009). Systematic Analysis of the Falcate-Spored *Graminicolous colletotrichum* and a Description of Six New Species from Warm-Season Grasses. *Mycologia*, *101*(5), 717-732. https://doi.org/10.3852/08-230

Crouch, J. A., Beirn, L. A., Cortese, L. M., Bonos, S. A., & Clarke, B. B. (2009). Anthracnose Disease of Switchgrass Caused by the Novel Fungal Species *Colletotrichum Navitas*. *Mycological Research*, *113*(12), 1411-1421. https://doi.org/https://doi.org/10.1016/j.mycres.2009.09.010

Crous, P. W., Groenewald, J. Z., Slippers, B., & Wingfield, M. J. (2016). Global Food and Fibre Security Threatened by Current Inefficiencies in Fungal Identification. *Philos Trans R Soc Lond B Biol Sci*, Philosophical Transactions of the Royal Society B: Biological Sciences *371*(1709). https://doi.org/10.1098/rstb.2016.0024

Curry, K. J., Abril, M., Avant, J. B., & Smith, B. J. (2002). Strawberry Anthracnose: Histopathology of *Colletotrichum acutatum* and *C. Fragariae*. *Phytopathology*, *92*(10), 1055-1063.

Damm, U., Cannon, P., Woudenberg, J., & Crous, P. (2012). The C*olletotrichum acutatum* Species Complex. *Studies in Mycology*, *73*, 37-113.

Damm, U., Sato, T., Alizadeh, A., Groenewald, J. Z., & Crous, P. W. (2019). The *Colletotrichum dracaenophilum, C. magnum* and *C. orchidearum* Species Complexes. *Studies in Mycology*, *92*, 1-46. https://doi.org/https://doi.org/10.1016/j.simyco.2018.04.001

David, S., Kirkby, R., & Kasozi, S. (2000). *Assessing the Impact of Bush Bean Varieties on Poverty Reduction in Sub-Saharan Africa: Evidence from Uganda*. Citeseer.

Davis, R., Irwin, J., Cameron, D., & Shepherd, R. (1987). Epidemiological Studies on the Anthracnose Diseases of *Stylosanthes* Spp. Caused by *Colletotrichum gloeosporioides* in North Queensland and Pathogenic Specialization within the Natural Fungal Populations. *Australian Journal of Agricultural Research*, *38*(6), 1019-1032.

De Ron, A. M., González, A. M., Rodiño, A. P., Santalla, M., Godoy, L., & Papa, R. (2016). History of the Common Bean Crop: Its Evolution Beyond Its Areas of Origin and Domestication. *Arbor*, *192*(a317).

De Silva, D. D., Crous, P. W., Ades, P. K., Hyde, K. D., & Taylor, P. W. (2017a). Life Styles of *Colletotrichum* Species and Implications for Plant Biosecurity. *Fungal Biology Reviews*, *31*(3), 155-168. https://doi.org/https://doi.org/10.1016/j.fbr.2017.05.001

de Silva, D. D., Groenewald, J. Z., Crous, P. W., Ades, P. K., Nasruddin, A., Mongkolporn, O., & Taylor, P. W. (2019a). Identification, Prevalence and Pathogenicity of *Colletotrichum* Species Causing Anthracnose of *Capsicum annuum* in Asia. *IMA Fungus*, *10*(1), 1-32. https://doi.org/10.1186/s43008-019-0001-y

Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., . . . Foster, G. D. (2012). The Top 10 Fungal Pathogens in Molecular Plant Pathology. *Molecular Plant Pathology*, *13*(4), 414-430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Debouck D.G. (1999). Diversity in *Phaseolus* Species in Relation to the Common Bean. In: Singh S.P. (Ed.). Common Bean Improvement in the Twenty-First Century, Pp. 25-52. Springer, the Netherlands.

Delgado-Salinas, A., Turley, T., Richman, A., & Lavin, M. (1999). Phylogenetic Analysis of the Cultivated and Wild Species of *Phaseolus* (Fabaceae). *Systematic Botany*, 438-460.

Diao, Y.-Z., Zhang, C., Liu, F., Wang, W.-Z., Liu, L., Cai, L., & Liu, X.-L. (2017). *Colletotrichum* Species Causing Anthracnose Disease of Chili in China. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *38*, 20.

dos Santos, G. R., de Castro Neto, M. D., de Almeida, H. S., Ramos, L. N., Sarmento, R. A., Lima, S. d. O., & Erasmo, E. A. L. (2009). Effect of Nitrogen Doses on Disease Severity and Watermelon Yield. *Horticultura brasileira*, *27*, 330-334.

Dou, D., & Zhou, J. M. (2012). Phytopathogen Effectors Subverting Host Immunity: Different Foes, Similar Battleground. *Cell Host Microbe*, *12*(4), 484-495. https://doi.org/10.1016/j.chom.2012.09.003

Douanla-Meli, C., & Unger, J.-G. (2017). Phylogenetic Study of the *Colletotrichum* Species on Imported Citrus Fruits Uncovers a Low Diversity and a New Species in the *Colletotrichum gigasporum* Complex. *Fungal biology*, *121*(10), 858-868.

Doyle, V. P., Oudemans, P. V., Rehner, S. A., & Litt, A. (2013a). Habitat and Host Indicate Lineage Identity in *Colletotrichum gloeosporioides* S.L. From Wild and Agricultural Landscapes in North America. *PLOS ONE*, *8*(5), e62394. https://doi.org/10.1371/journal.pone.0062394

Dromph, K. M., & Borgen, A. (2001). Reduction of Viability of Soil Borne Inoculum of Common Bunt (*Tilletia tritici*) by Collembolans. *Soil Biology and Biochemistry*, *33*(12-13), 1791-1795.

Du, M., Schardl, C. L., Nuckles, E. M., & Vaillancourt, L. J. (2005). Using Mating-Type Gene Sequences for Improved Phylogenetic Resolution of *Collectotrichum* Species Complexes. *Mycologia*, *97*(3), 641-658.

Edje, O., Mughogho, L., Rao, Y., & Msuku, W. (1980). Bean Production in Malawi. Potential for Field Beans in Eastern Africa, Proceedings of a Workshop held in Lilongwe, Malawi,

Ekefan, E., Simons, S., & Nwankiti, A. (2000). Survival of *Colletotrichum gloeosporioides* (Causal Agent of Yam Anthracnose) in Soil. *Tropical Science*, *40*(4), 163-168.

Estrada, A., Dodd, J. C., & Jeffries, P. (1992). Effects of Environment on the in Vitro Growth and Development of *Colletotrichum gloeosporioides* Isolates from the Philippines. IV International Mango Symposium 341,

Everett, K., Pushparajah, I., Timudo, O., Chee, A. A., Scheper, R., Shaw, P., . . . Wood, P. (2018). Infection Criteria, Inoculum Sources and Splash Dispersal Pattern of *Colletotrichum acutatum* Causing Bitter Rot of Apple in New Zealand. *European Journal of Plant Pathology*, *152*(2), 367-383.

FAOSTAT. (2021). *Faostat Crop Statistics. Available Online: Http://Faostat.Fao.Org/Site/567/Desktopdefault.Aspx* Retrieved 20 August from

Farr, D. F. R., A.Y. (2019). Fungal Databases. *Systematic Mycology and Microbiology Laboratory; ARS, USDA:*(Washington, DC, USA).

Freeman, S., Horowitz-Brown, S., Afanador-Kafuri, L., Maymon, M., & Minz, D. (2008). *Colletotrichum*: Host Specificity and Pathogenicity on Selected Tropical and Subtropical Crops. IV International Symposium on Tropical and Subtropical Fruits 975,

Freeman, S., Katan, T., & Shabi, E. (1998). Characterization of *Colletotrichum* Species Responsible for Anthracnose Diseases of Various Fruits. *Plant Disease*, *82*(6), 596-605.

Freeman, S., & Shabi, E. (1996). Cross-Infection of Subtropical and Temperate Fruits By *Colletotrichum* species from Various Hosts. *Physiological and Molecular Plant Pathology*, *49*(6), 395-404. https://doi.org/https://doi.org/10.1006/pmpp.1996.0062

Freeman, S., Shalev, Z., & Katan, J. (2002). Survival in Soil of *Colletotrichum acutatum* and *C. gloeosporioides* Pathogenic on Strawberry. *Plant Disease*, *86*(9), 965-970.

Gan, P., Ikeda, K., Irieda, H., Narusaka, M., O'Connell, R. J., Narusaka, Y., . . . Shirasu, K. (2013). Comparative Genomic and Transcriptomic Analyses Reveal the Hemibiotrophic Stage Shift of Colletotrichum Fungi. *New Phytologist*, *197*(4), 1236-1249.

Gan, P., Narusaka, M., Kumakura, N., Tsushima, A., Takano, Y., Narusaka, Y., & Shirasu, K. (2016). Genus-Wide Comparative Genome Analyses of *Colletotrichum* Species Reveal Specific Gene Family Losses and Gains During Adaptation to Specific Infection Lifestyles. *Genome Biology and Evololution*, *8*(5), 1467-1481. https://doi.org/10.1093/gbe/evw089

Gatsby. (2014). Uganda Bean Production (Naro).

Gaudencia J. Kiptoo, M. G. K., Oliver K. Kiplagat, Lexa G. Matasyoh,. (2020). Incidence and Severity of Anthracnose (*Colletotrichum lindemuthianum*) on Selected Common Bean (*Phaseolus vulgaris L.*) Genotypes *American Journal of Agricultural Research*, *5:84*. https://doi.org/DOI:10.28933/ajar-2019-09-2705

Gautam, A. (2014). The Genera *Colletotrichum:* An Incitant of Numerous New Plant Diseases in India. *Journal on New Biological Reports*, *3*(1), 9-21.

Giblin, F., Coates, L., & Irwin, J. (2010). Pathogenic Diversity of Avocado and Mango Isolates of *Colletotrichum gloeosporioides* Causing Anthracnose and Pepper Spot in Australia. *Australasian Plant Pathology*, *39*(1), 50-62.

Gomes, R., Glienke, C., Videira, S., Lombard, L., Groenewald, J., & Crous, P. (2013). Diaporthe: A Genus of Endophytic, Saprobic and Plant Pathogenic Fungi. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *31*, 1.

González, E., Sutton, T. B., & Correll, J. C. (2006a). Clarification of the Etiology of *Glomerella* Leaf Spot and Bitter Rot of Apple Caused by *Colletotrichum* Spp. Based on Morphology and Genetic, Molecular, and Pathogenicity Tests. *Phytopathology*, *96*(9), 982-992. https://doi.org/10.1094/phyto-96-0982

Guarnaccia, V., Groenewald, J., Polizzi, G., & Crous, P. W. (2017). High Species Diversity in *Colletotrichum* Associated with Citrus Diseases in Europe. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *39*, 32.

Gunnell, P. S., & Gubler, W. D. (1992). Taxonomy and Morphology of *Colletotrichum* Species Pathogenic to Strawberry. *Mycologia*, *84*(2), 157-165.

Guyon, K., Balagué, C., Roby, D., & Raffaele, S. (2014). Secretome Analysis Reveals Effector Candidates Associated with Broad Host Range Necrotrophy in the Fungal Plant Pathogen *Sclerotinia sclerotiorum*. *BMC genomics*, *15*(1), 1-19.

Hadden, J. F. (1989). *The Etiology and Control of Pepper Anthracnose*. Louisiana State University and Agricultural & Mechanical College.

Hamada, N., & De Mio, L. M. (2017). Survival of Pathogenic *Colletotrichum* Isolates on Dormant Buds, Twigs and Fallen Leaves of Apple Trees in Commercial Orchards. *Fruits*, *72*(3), 158-165.

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., . . . Sessitsch, A. (2015). The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiol Mol Biol Rev*, *79*(3), 293-320. https://doi.org/10.1128/mmbr.00050-14

Harp, T. L., Pernezny, K., Ivey, M. L. L., Miller, S. A., Kuhn, P. J., & Datnoff, L. (2008). The Etiology of Recent Pepper Anthracnose Outbreaks in Florida. *Crop Protection*, *27*(10), 1380-1384.

Hassan, O., Jeon, J. Y., Chang, T., Shin, J. S., Oh, N. K., & Lee, Y. S. (2018). Molecular and Morphological Characterization of *Colletotrichum* Species in the *Colletotrichum gloeosporioides* Complex Associated with Persimmon Anthracnose in South Korea. *Plant Disease*, *102*(5), 1015-1024.

Henderson, C., Cripps, M., & Casonato, S. (2019). Distribution of *Puccinia Punctiformis* in above-Ground Tissue of *Cirsium arvense* (Californian Thistle). *New Zealand Plant Protection*, *72*, 265-270.

Hiruma, K., Gerlach, N., Sacristán, S., Nakano, R. T., Hacquard, S., Kracher, B., . . . O'Connell, R. J. (2016). Root Endophyte *Colletotrichum tofieldiae* Confers Plant Fitness Benefits That Are Phosphate Status Dependent. *Cell*, *165*(2), 464-474.

Hofer, K. M., Braithwaite, M., Braithwaite, L. J., Sorensen, S., Siebert, B., Pather, V., . . . Toome-Heller, M. (2021). First Report of *Colletotrichum fructicola*, C. Perseae, and C. Siamense Causing Anthracnose Disease of Avocado (Persea Americana) in New Zealand. *Plant Disease* https://doi.org/10.1094/pdis-06-20-1313-pdn

Hu, M.-J., Grabke, A., & Schnabel, G. (2015). Investigation of the *Colletotrichum gloeosporioides* Species Complex Causing Peach Anthracnose in South Carolina. *Plant Disease*, *99*(6), 797-805.

Huang, F., Chen, G., Hou, X., Fu, Y., Cai, L., Hyde, K., & Li, H. (2013). *Colletotrichum* Species Associated with Cultivated Citrus in China. *Fungal Diversity*, *61*(1), 61-74.

Hyde, K. D., Cai, L., McKenzie, E., Yang, Y., Zhang, J., & Prihastuti, H. (2009). *Colletotrichum*: A Catalogue of Confusion. *Fungal Diversity*, *39*(1), 1-17.

Idnurm, A., Verma, S., & Corrochano, L. M. (2010). A Glimpse into the Basis of Vision in the Kingdom Mycota. *Fungal Genetics and Biology*, *47*(11), 881-892. https://doi.org/10.1016/j.fgb.2010.04.009

Ishikawa, F. H., Barcelos, Q. L., Alves, E., Camargo Jr, O. A., & De Souza, E. A. (2010). Symptoms and Prepenetration Events Associated with the Infection of Common Bean by the Anamorph and Teleomorph of *Glomerella Cingulata* F. Sp. *Phaseoli. Journal of Phytopathology*, *158*(4), 270-277.

Jayasinghe, C., & Fernando, T. (2009). First Report of *Colletotrichum acutatum* on *Mangifera indica* in Sri Lanka. *Ceylon Journal of Science (Biological Sciences)*, *38*(1).

Jayawardena, R., Hyde, K., Jeewon, R., Li, X., Liu, M., & Yan, J. (2016). Mycosphere Essay 6: Why Is It Important to Correctly Name *Colletotrichum* Species? Mycosphere 7, 1076-1092

Jeffries, P., Dodd, J. C., Jeger, M. J. and Plumley, R. A. . (1990). The Biology and Control of *Colletotrichum* Species on Tropical Fruit Crops. *Plant Pathology*, *39*, 343–366. .

Johnston, P. R., & Jones, D. (1997). Relationships among *Colletotrichum* Isolates from Fruit-Rots Assessed Using Rdna Sequences. *Mycologia*, *89*(3), 420-430.

Jones, M. D., Forn, I., Gadelha, C., Egan, M. J., Bass, D., Massana, R., & Richards, T. A. (2011). Discovery of Novel Intermediate Forms Redefines the Fungal Tree of Life. *Nature*, *474*(7350), 200-203.

Kamfwa, K., Cichy, K. A., & Kelly, J. D. (2019). Identification of Quantitative Trait Loci for Symbiotic Nitrogen Fixation in Common Bean. *Theoretical and Applied Genetics*, *132*(5), 1375-1387.

Kamle, M., Pandey, B., Kumar, P., & Muthu Kumar, M. (2013). A Species-Specific PCR Based Assay for Rapid Detection of Mango Anthracnose Pathogen *Colletotrichum gloeosporioides* Penz. And Sacc. *Journal of Plant Pathology and Microbiology*, *4*(6).

Kang, B. K., Kim, J., Lee, K. H., Lim, S. C., Ji, J. J., Lee, J. W., & Kim, H. T. (2009). Effects of Temperature and Moisture on the Survival of *Colletotrichum acutatum*, the Causal Agent of Pepper Anthracnose in Soil and Pepper Fruit Debris. *The Plant Pathology Journal*, *25*(2), 128-135.

Kenny, M., Galea, V., & Price, T. (2012). Germination and Growth of *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* Isolates from Coffee in Papua New Guinea and Their Pathogenicity to Coffee Berries. *Australasian Plant Pathology*, *41*(5), 519-528.

Khan, A., & Hsiang, T. (2003). The Infection Process of *Colletotrichum graminicola* and Relative Aggressiveness on Four Turfgrass Species. *Canadian Journal of Microbiology*, *49*(7), 433-442.

Khanzada, M. A., Ansari, A., Rajput, M. A., Maitlo, S., Rajput, A. Q., & Ujjan, A. A. (2018). Effect of Different Abiotic Factors on the Growth and Sporulation of *Colletotrichum gloeosporioides* Causing Anthracnose of Mango. *Plant Protection*, *2*(1), 23-30.

Kim, W. G., Cho, E. K., & Lee, E. J. (1986). Two Strains of *Colletotrichum Gloeosporioides* Penz. Causing Anthracnose on Pepper Fruits. *Korean Journal Plant Pathology*, *2*(2), 107-113.

Kivlin, S. N., Emery, S. M., & Rudgers, J. A. (2013). Fungal Symbionts Alter Plant Responses to Global Change. *American Journal of Botany*, *100*(7), 1445-1457.

Kleemann, J., Rincon-Rivera, L., Takahara, H., Neumann, U., & van Themaat, E. (2012). Van Der Does Hc, Hacquard S, Stüber K, Will I, Schmalenbach W, Schmelzer E, O'connell Rj. Sequential Delivery of Host-Induced Virulence Effectors by Appressoria and Intracellular Hyphae of the Phytopathogen *Colletotrichum higginsianum*. *PLoS Pathog*, *8*, e1002643.

Kluth, S., Kruess, A., & Tscharntke, T. (2002). Insects as Vectors of Plant Pathogens: Mutualistic and Antagonistic Interactions. *Oecologia*, *133*(2), 193-199. https://doi.org/10.1007/s00442-002-1016-3

Kogel, K.-H., Franken, P., & Hückelhoven, R. (2006). Endophyte or Parasite–What Decides? *Current Opinion in Plant Biology*, *9*(4), 358-363.

Kommula, S. K., Reddy, G. P. D., Undrajavarapu, P., & Kanchana, K. S. (2017). Effect of Various Factors (Temperature, Ph and Light Inten-835 Sity) on Growth of *Colletotrichum* Capsici Isolated from Infected Chilli. *International Journal of Pure and Applied Bioscience*, *5*, 535-543.

Kumar, D. S. S., & Hyde, K. D. (2004). Biodiversity and Tissue-Recurrence of Endophytic Fungi in Tripterygium Wilfordii. *Fungal Diversity*. *14*(7), 119-128.

Kumara, K., & Rawal, R. (2010). Influence of Carbon, Nitrogen, Temperature and Ph on the Growth and Sporulation of Some Indian Isolates of *Colletotrichum gloeosporioides* Causing Anthracnose Disease of Papaya (Carrica Papaya L). *Tropical Agricultural Research and Extension*, *11*.

Kumari, A. S. P. S. (2002). Survival of *Colletotrichum gloeosporioides*, the Causal Organism of Anthracnose Disease of Black Pepper. *Journal of Spices and Aromatic Crops*, *11*(2), 129-131. https://updatepublishing.com/journal/index.php/josac/article/view/4730

Kumari, P., Singh, R., & Punia, R. (2017). Survival of *Colletotrichum gloeosporioides* Causing Anthracnose of Mango at Different Depths and Durations in Soil. *Journal of Pharmacognosy and Phytochemistry*, *6*, 2194-2198.

Kutoš, T., Golob, T., Kač, M., & Plestenjak, A. (2003). Dietary Fibre Content of Dry and Processed Beans. *Food chemistry*, *80*(2), 231-235.

Latunde-Dada, A., Bailey, J., & Lucas, J. (1997). Infection Process of *Colletotrichum destructivum O'gara* from Lucerne (Medicago Sativa L.). *European Journal of Plant Pathology*, *103*(1), 35-41.

Lax, A., Templeton, G., & Meyer, W. (1985). Isolation, Purification, and Biological Activity of a Self-Inhibitor from Conidia of *Colletotrichum gloeosporioides*. *Phytopathology*, *75*(4), 386-390.

Leite, B., & Nicholson, R. L. (1992). Mycosporine-Alanine: A Self-Inhibitor of Germination from the Conidial Mucilage of *Colletotrichum Graminicola*. *Experimental Mycology*, *16*(1), 76-86.

León, A. M. M., Hernández, W. S., Pérez, L. G., Crespo, K., & Guarnaccia, V. (2018). First Report of Leaf Anthracnose Caused by *Colletotrichum grossum* on Mango (*Mangifera Indica*) in Cuba. *Journal of Plant Pathology*, *1pp*(1).

Liu, F., Tang, G., Zheng, X., Li, Y., Sun, X., Qi, X., . . . Chang, X. (2016). Molecular and Phenotypic Characterization of *Colletotrichum* Species Associated with Anthracnose Disease in Peppers from Sichuan Province, China. *Scientific reports*, *6*(1), 1-17.

Lu, G., Cannon, P. F., Reid, A., & Simmons, C. M. (2004). Diversity and Molecular Relationships of Endophytic *Colletotrichum* Isolates from the Iwokrama Forest Reserve, Guyana. *Mycological Research*, *108*(1), 53-63.

Mahmodi, F. (2013). *Morphological, Molecular Characterization and Pathogenicity of Colletotrichum Spp. Isolated from Legume Crops in Peninsular Malaysia* (Doctoral dissertation, Universiti Putra Malaysia).

Manandhar, J., Hartman, G., & Sinclair, J. (1986). *Colletotrichum Destructivum*, the Anamorph of Glomerella Glycines. *Phytopathology*, *76*(3), 282-285.

Marcenaro, D., & Valkonen, J. P. T. (2016). Seedborne Pathogenic Fungi in Common Bean (*Phaseolus vulgaris* Cv. Inta Rojo) in Nicaragua. *PloS ONE*, *11*(12), e0168662. https://doi.org/10.1371/journal.pone.0168662

Marin-Felix, Y., Groenewald, J., Cai, L., Chen, Q., Marincowitz, S., Barnes, I., . . . Damm, U. (2017). Genera of Phytopathogenic Fungi: Gophy 1. *Studies in Mycology*, *86*, 99-216. https://doi.org/10.1016/j.simyco.2017.04.002

Marvel, J., Alexander, S., & Stromberg, E. (2003). Characterization of Pepper Anthracnose Isolates from the Eastern Shore of Virginia and Eastern US. *Phytopathology*, *93*, S58.

McClean, P. E., Lavin, M., Gepts, P., & Jackson, S. A. (2008). *Phaseolus vulgaris*: A Diploid Model for Soybean. In *Genetics and Genomics of Soybean* (pp. 55-76). Springer.

Mello, A. F. S., Machado, A. C. Z., & Bedendo, I. P. (2004). Development of *Colletotrichum gloeosporioides* Isolated from Green Pepper in Different Culture Media, Temperatures, and Light Regimes. *Scientia Agricola*, *61*(5), 542-544.

Meshram, M. C., Guldekar, D., & Potdukhe, S. (2014). Morphological Character of *Colletotrichum gloeosporioides* from Various Hosts. *Journal of Soils and Crops*, *24*(2), 275-280.

Mo, J., Zhao, G., Li, Q., Solangi, G. S., Tang, L., Guo, T., . . . Hsiang, T. (2018). Identification and Characterization of *Colletotrichum* Species Associated with Mango Anthracnose in Guangxi, China. *Plant Disease*, *102*(7), 1283-1289.

Mordue, J. (1971). *Colletotrichum capsici.* [Descriptions of Fungi and Bacteria]. *IMI Descriptions of Fungi and Bacteria*(32).

Morkeliūnė, A., Rasiukevičiūtė, N., & Valiuškaitė, A. (2021). Meteorological Conditions in a Temperate Climate for *Colletotrichum acutatum*, Strawberry Pathogen Distribution and Susceptibility of Different Cultivars to Anthracnose. *Agriculture*, *11*(1), 80.

Mourice, S. K., & Tryphone, G. M. (2012). Evaluation of Common Bean (*Phaseolus Vulgaris L.*) Genotypes for Adaptation to Low Phosphorus. *ISRN Agronomy*, *2012*.

Münch, S., Lingner, U., Floss, D. S., Ludwig, N., Sauer, N., & Deising, H. B. (2008). The Hemibiotrophic Lifestyle of *Colletotrichum* Species. *Journal of Plant Physiology*, *165*(1), 41-51.

Munir, Amsden, B., Dixon, E., Vaillancourt, L., & Gauthier, N. A. W. (2016a). Characterization of Colletotrichum Species Causing Bitter Rot of Apple in Kentucky Orchards. *Plant Dis*, *100*(11), 2194-2203. https://doi.org/10.1094/pdis-10-15-1144-re

Nagamani. (2009). *Handbook of Soil Fungi* (Vol. ISBN 10: 818823771X / ISBN 13: 9788188237715). I K International, New Delhi,.

Nelson. (2008a). Anthracnose of Avocado. *Plant Disease. PD-58. Available online: https://www.ctahr.hawaii.edu/oc/freepubs/pdf/pd-58.pdf (accessed on 15 September 2021).*

Nelson, S. C. (2008b). Mango Anthracnose (*Colletotrichum gloeosporiodes*). University of Hawai'i at

Mānoa, College of Tropical Agriculture and Human Resources, 42(7), 54-59.

Norman, D., & Strandberg, J. (1997). Survival of *Colletotrichum acutatum* in Soil and Plant Debris of Leatherleaf Fern. *Plant Disease*, *81 10*, 1177-1180.

Ntahimpera, N., Madden, L. V., & Wilson, L. L. (1997). Effect of Rain Distribution Alteration on Splash Dispersal of *Colletotrichum acutatum*. *Phytopathology*, *87*(6), 649-655. https://doi.org/10.1094/phyto.1997.87.6.649

O'Connell, R. J., Thon, M. R., Hacquard, S., Amyotte, S. G., Kleemann, J., Torres, M. F., . . . Alkan, N. (2012). Lifestyle Transitions in Plant Pathogenic *Colletotrichum* Fungi Deciphered by Genome and Transcriptome Analyses. *Nature Genetics*, *44*(9), 1060-1065.

Obanor, F. O. (2006). *Olive Leaf Spot: Epidemiology and Control. PhD thesis,* Lincoln University.

Okoth, P., Okoth, S., & Jefwd, J. M. (2013). Commission on Genetic Resources for Food and Agriculture. International Centre for Tropical Agriculture (CIAT), 234(4), 232-279.

Osorio-Díaz, P., Bello-Pérez, L. A., Sáyago-Ayerdi, S. G., Benítez-Reyes, M. d. P., Tovar, J., & Paredes-López, O. (2003). Effect of Processing and Storage Time on in Vitro Digestibility and Resistant Starch Content of Two Bean (*Phaseolus vulgaris L*) Varieties. *Journal of the Science of Food and Agriculture*, *83*(12), 1283-1288.

The Pan-Africa Bean Research Alliance's (PABRA), C. (2016). *First Drought-Resilient High Iron Beans for Uganda Released* The Legumes Research Program*,* National Crops Resources Research Institute (NaCRRI)*,* Uganda*,* 32(2), 21-34.

Retrieved August, 24 from https://blog.ciat.cgiar.org/first-drought-resilient-high-iron-beans-for-uganda-released/

Padder, B., Sharma, P., Awale, H., & Kelly, J. (2017). *Colletotrichum lindemuthianum*, the Causal Agent of Bean Anthracnose. *Journal of Plant Pathology*, 317-330.

Padder, B., Sharma, P., Sharma, O., & Kapoor, V. (2007). Genetic Diversity and Gene Flow Estimates among Five Populations of *Colletotrichum lindemuthianum* across Himachal Pradesh. *Physiological and Molecular Plant Pathology*, *70*(1-3), 8-12.

Pardo-De la Hoz, C., Calderón, C., Rincón, A., Cárdenas, M., Danies, G., López-Kleine, L., . . . Jiménez, P. (2016). Species from the *Colletotrichum acutatum*, *Colletotrichum boninense* and *Colletotrichum gloeosporioides* Species Complexes Associated with Tree Tomato and Mango Crops in Colombia. *Plant Pathology*, *65*(2), 227-237.

Parikka, P., & Lemmetty, A. (2010). Survival of *Colletorichum acutatum* on Alternate Hosts. XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 926,

Parikka, P., Lemmetty, A., Sundelin, T., Strømeng, G., & Stensvand, A. (2014). Survival of *Colletotrichum acutatum* in Plant Residue. XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): II 1117,

Pastor Corrales, M. A., & Tu, J. (1989). Anthracnose. In. International Center for Tropical Agriculture.

Pathania, A., Sharma, S. K., & Sharma, P. N. (2014). Common Bean. In *Broadening the Genetic Base of Grain Legumes* (pp. 11-50). Springer.
Pavitra Kumari, R., & Singh, R. (2017). Anthracnose of Mango Incited by *Colletotrichum gloeosporioides*: A Comprehensive Review. *International Journal of Pure and Applied Bioscience*, *5*(1), 48-56.
Peres, N., Timmer, L., Adaskaveg, J., & Correll, J. (2005). Lifestyles of *Colletotrichum acutatum*. *Plant Disease*, *89*(8), 784-796.
Perfect, S. E., Hughes, H. B., O'Connell, R. J., & Green, J. R. (1999). *Colletotrichum*: A Model Genus for Studies on Pathology and Fungal-Plant Interactions. *Fungal Genet Biol*, *27*(2-3), 186-198. https://doi.org/10.1006/fgbi.1999.1143
Photita, W., Lumyong, S., Lumyong, P., McKenzie, E., & Hyde, K. (2004). Are Some Endophytes of Musa Acuminata Latent Pathogens? *Fungal Diversity*.
Phoulivong, S., McKenzie, E., & Hyde, K. (2012). Cross Infection of *Colletotrichum* Species; a Case Study with Tropical Fruits. *Current Research in Environmental & Applied Mycology*, *2*(2), 99-111.
Pinto, J., Pereira, R., Mota, S., Ishikawa, F., & Souza, E. (2012). Investigating Phenotypic Variability in *Colletotrichum lindemuthianum* Populations. *Phytopathology*, *102*(5), 490-497.
Platt, B. (1962). Proteins in Nutrition. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *156*(964), 337-344.
Ploetz, R. C., Benscher, D., Vazquez, A., Colls, A., Nagel, J., & Schaffer, B. (1996). A Re-examination of Mango Decline in Florida. *Plant Disease, 80,* 664-668.
Ponte, J. J. d. (1996). Clinica De Doencas De Plantas. *Fortaleza-CE: UFC*, pp. 871.
Porras-Alfaro, A., & Bayman, P. (2011). Hidden Fungi, Emergent Properties: Endophytes and Microbiomes. *Annual Review of Phytopathology*, *49*, 291-315.
Promputtha, I., Lumyong, S., Dhanasekaran, V., McKenzie, E. H. C., Hyde, K. D., & Jeewon, R. (2007). A Phylogenetic Evaluation of Whether Endophytes Become Saprotrophs at Host Senescence. *Microbial ecology*, *53*(4), 579-590.
Prusky, D. (1996). Pathogen Quiescence in Postharvest Diseases. *Annual Review of Phytopathology*, *34*(1), 413-434.
Prusky, D., Alkan, N., Mengiste, T., & Fluhr, R. (2013). Quiescent and Necrotrophic Lifestyle Choice During Postharvest Disease Development. *Annual review of phytopathology*, *51*, 155-176.
Purschwitz, J., Müller, S., Kastner, C., & Fischer, R. (2006). Seeing the Rainbow: Light Sensing in Fungi. *Current Opinion in Microbiology*, *9*(6), 566-571. https://doi.org/10.1016/j.mib.2006.10.011
Qin, L., Zhang, Y., Su, Q., Chen, Y., Nong, Q., Xie, L., . . . Huang, S. (2019). First Report of Anthracnose of Mangifera Indica Caused by *Colletotrichum scovillei* in China. *Plant Disease*, *103*(5), 1043.
Ranathunge, N., Mongkolporn, O., Ford, R., & Taylor, P. (2012). *Colletotrichum truncatum* Patho-system on *Capsicum* Spp: Infection, Colonization and Defence Mechanisms. *Australasian Plant Pathology*, *41*(5), 463-473.
Rayner, R. W. (1970). A Mycological Colour Chart. Commonwealth Mycological Institute (Great Britain); British Mycological Society.
Rex, D., Schimmelpfennig, S., Jansen-Willems, A., Moser, G., Kammann, C., & Müller, C. (2015). Microbial Community Shifts 2.6 Years after Top Dressing of Miscanthus Biochar, Hydrochar and Feedstock on a Temperate Grassland Site. *Plant and Soil*, *397*(1-2), 261-271.
Ripoche, A., Jacqua, G., Bussière, F., Guyader, S., & Sierra, J. (2008). Survival of *Colletotrichum gloeosporioides* (Causal Agent of Yam Anthracnose) on Yam Residues Decomposing in Soil. *Applied Soil Ecology*, *38*(3), 270-278.
Rispail, N., Kaló, P., Kiss, G. B., Ellis, T. N., Gallardo, K., Thompson, R. D., . . . González, E. M. (2010). Model Legumes Contribute to Faba Bean Breeding. *Field Crops Research*, *115*(3), 253-269.
Rivera-Orduña, F. N., Suarez-Sanchez, R. A., Flores-Bustamante, Z. R., Gracida-Rodriguez, J. N., & Flores-Cotera, L. B. (2011). Diversity of Endophytic Fungi of Taxus Globosa (Mexican Yew). *Fungal Diversity*, *47*(1), 65-74.

Rodríguez-Guerra, R., Ramírez-Rueda, M. T., Cabral-Enciso, M., García-Serrano, M., Lira-Maldonado, Z., Guevara-González, R. G., . . . Simpson, J. (2005). Heterothallic Mating Observed between Mexican Isolates of *Glomerella lindemuthiana*. *Mycologia*, *97*(4), 793-803. https://doi.org/10.3852/mycologia.97.4.793

Rojas, E. I., Rehner, S. A., Samuels, G. J., Van Bael, S. A., Herre, E. A., Cannon, P., . . . Zhang, Y. (2010). *Colletotrichum gloeosporioides* Sl Associated with Theobroma Cacao and Other Plants in Panama: Multilocus Phylogenies Distinguish Host-Associated Pathogens from Asymptomatic Endophytes. *Mycologia*, *102*(6), 1318-1338.

Samuelian, S. K., Greer, L., Savocchia, S., & Steel, C. C. (2012). Overwintering and Presence of *Colletotrichum acutatum* (Ripe Rot) on Mummified Bunches, Dormant Wood, Developing Tissues and Mature Berries Of. *Vitis*, *51*(1), 33-37.

Sanabria, A., Mahuku, G., Kelemu, S., Cadavid, M., García, C., Hío, J. C., . . . Osorio, J. A. (2010). Molecular Identification and Characterization of *Colletotrichum* Spp Isolates from Tahiti Lime, Tamarillo, and Mango. *Agronomía Colombiana*, *28*(3), 383-391.

Sanders, G. M., & Korsten, L. (2003). A Comparative Morphological Study of South African Avocado and Mango Isolates of *Colletotrichum gloeosporioides*. *Canadian Journal of Botany*, *81*(8), 877-885.

Sanders, G. M., Korsten, L., & Wehner, F. (2000). Survey of Fungicide Sensitivity in *Colletotrichum gloeosporioides* from Different Avocado and Mango Production Areas in South Africa. *European Journal of plant pathology*, *106*(8), 745-752.

Sangeetha, C., & Rawal, R. (2010). Temperature Requirement of Different Isolates of *Colletotrichum gloeosporioides* Isolated from Mango. *African Journal of Biotechnology*, *9*(21), 3086-3090.

Schwartz, H., & Pastor-Corrales, M. (1994). Problemas De Producción Del Fríjol En Los Trópicos. In: Cali, Colombia: Centro Internacional de Agricultura Tropical.

Schwartz, H., & Pastor-Corrales, M. (2005). Anthracnose. *Compendium of bean diseases*, *25*, 27.

Serrano, M., Coluccia, F., Torres, M., L'Haridon, F., & Métraux, J.-P. (2014). The Cuticle and Plant Defense to Pathogens. *Frontiers in Plant Science*, *5*, 274.

Shafi, A. (2016). *Botryosphaeria Dieback in Vineyards: Conidial Dispersal, Infection, Disease Development and Control. PhD thesis* Lincoln University].

Sharma, Maymon, M., & Freeman, S. (2017). Epidemiology, Pathology and Identification of *Colletotrichum* Including a Novel Species Associated with Avocado (Persea Americana) Anthracnose in Israel. *Scientific Reports*, *7*(1), 15839. https://doi.org/10.1038/s41598-017-15946-w

Sharma, G., Kumar, N., Weir, B. S., Hyde, K. D., & Shenoy, B. D. (2013). The Apmat Marker Can Resolve *Colletotrichum* Species: A Case Study with *Mangifera Indica. Fungal Diversity*, *61*(1), 117-138. https://doi.org/10.1007/s13225-013-0247-4

Sharma, M., & Kulshrestha, S. (2015). *Colletotrichum gloeosporioides*: An Anthracnose Causing Pathogen of Fruits and Vegetables. *Biosciences Biotechnology Research Asia*, *12*(2), 1233-1246.

Sharma, P., Sharma, O., Padder, B., & Kapil, R. (2008). Yield Loss Assessment in Common Bean Due to Anthracnose (*Colletotrichum lindemuthianum*) under Sub Temperate Conditions of North-Western Himalayas. *Indian Phytopathology*, *61*(3), 323.

Shaw, M. W., Emmanuel, C. J., Emilda, D., Terhem, R. B., Shafia, A., Tsamaidi, D., . . . Van Kan, J. A. (2016). Analysis of Cryptic, Systemic Botrytis Infections in Symptomless Hosts. *Frontiers in Plant Science*, *7*, 625.

Shi, A., Kantartzi, S., Mmbaga, M., Chen, P., Mrema, F., & Nnodu, E. (2008). Pcr-Based Markers for Detection of *Colletotrichum acutatum* and *C. gloeosporioides* in Flowering Dogwood (Cornus Florida). *Australasian Plant Pathology*, *37*(1), 65-68.

Silva-Rojas, & Ávila-Quezada. (2011). Phylogenetic and Morphological Identification of *Colletotrichum boninense*: A Novel Causal Agent of Anthracnose in Avocado. *Plant Pathology*, *60*(5), 899-908.

Singh, S. P. (2001). Broadening the Genetic Base of Common Bean Cultivars: A Review. *Crop science*, *41*(6), 1659-1675.
Soltani, J., Haghighi, M. Y.-P., & Nazeri, S. (2014). Light, Temperature, and Aging Dependent Vegetative Growth and Sporulation of *Colletotrichum gloeosporioides* on Different Culture Media. *Journal of Medicinal Plants Research*, *8*(4), 208-216.
Sreenivasaprasad, S., & Talhinhas, P. (2005). Genotypic and Phenotypic Diversity in *Colletotrichum acutatum*, a Cosmopolitan Pathogen Causing Anthracnose on a Wide Range of Hosts. *Molecular Plant Pathology*, *6*(4), 361-378.
Stone, J., Maloy, O., & Murray, T. (2001). Encyclopedia of Plant Pathology. In: New York, NY: Wiley.
Sutton, B. (1992). The Genus *Glomerella* and Its Anamorph *Colletotrichum*. *The genus Glomerella and its anamorph Colletotrichum.*, 1-26.
Sutton, T., & Shane, W. (1983). Epidemiology of the Perfect Stage of *Glomerella Cingulata* on Apples. *Phytopathology*, *73*(8), 1179-1183.
Takushi, T. (2015). Survival Period of *Colletotrichum gloeosporioides*, Causal Agent of Mango Anthracnose, on Diseased Withered Leaves of Mango at Different Temperatures. *Kyushu Plant Protection Research*, *61*, 16-19.
Talhinhas, P., Mota-Capitão, C., Martins, S., Ramos, A. P., Neves-Martins, J., Guerra-Guimarães, L., . . . Oliveira, H. (2011). Epidemiology, Histopathology and Aetiology of Olive Anthracnose Caused by *Colletotrichum acutatum* and *C. gloeosporioides* in Portugal. *Plant Pathology*, *60*(3), 483-495. https://doi.org/https://doi.org/10.1111/j.1365-3059.2010.02397.x
Tang, A. M., Hyde, K. D., & Corlett, R. T. (2003). Diversity of Fungi on Wild Fruits in Hong Kong. *Fungal Diversity,* 14,165-185
Tisch, D., & Schmoll, M. (2010). Light Regulation of Metabolic Pathways in Fungi. *Applied Microbiology and Biotechnology*, *85*(5), 1259-1277. https://doi.org/10.1007/s00253-009-2320-1
Tu, J. (1983). Epidemiology of Anthracnose Caused by *Colletotrichum lindemuthianum* on White Bean (*Phaseolus vulgaris*) in Southern Ontario: Survival of the Pathogen. *Plant Disease*, *67*(4), 402-404.
Udayanga, D., Manamgoda, D. S., Liu, X., Chukeatirote, E., & Hyde, K. D. (2013). What Are the Common Anthracnose Pathogens of Tropical Fruits? *Fungal Diversity*, *61*(1), 165-179.
Uganda Investment Authority (UIA). (2010). *Mango Production in Uganda*. https://www.ugandainvest.com
Vaillancourt, L., Wang, J., & Hanau, R. (2000). Genetic Regulation of Sexual Compatibility in *Glomerella Graminicola*. In (pp. 29-44): APS Press, St Paul, USA.
van Kan, J. A. (2006). Licensed to Kill: The Lifestyle of a Necrotrophic Plant Pathogen. *Trends in Plant Science*, *11*(5), 247-253.
Van Kan, J. A., Shaw, M. W., & Grant-Downton, R. T. (2014). Botrytis Species: Relentless Necrotrophic Thugs or Endophytes Gone Rogue? *Molecular Plant Pathology*, *15*(9), 957-961.
Varzea, V., Rodrigues Jr, C., & Lewis, B. (2002). Distinguishing Characteristics and Vegetative Compatibility of *Colletotrichum kahawe* in Comparison with Other Related Species from Coffee. *Plant Pathology*, *51*(2), 202-207.
Vieira, W. A., Michereff, S. J., de Morais, M. A., Hyde, K. D., & Câmara, M. P. (2014). Endophytic Species of *Colletotrichum* Associated with Mango in Northeastern Brazil. *Fungal Diversity*, *67*(1), 181-202.
Voegele, R. T., & Mendgen, K. W. (2011). Nutrient Uptake in Rust Fungi: How Sweet Is Parasitic Life? *Euphytica*, *179*(1), 41-55.
Weir, B., Johnston, P., & Damm, U. (2012~~a~~). The *Colletotrichum gloeosporioides* Species Complex. *Studies in Mycology*, *73*, 115-180. https://doi.org/10.3114/sim0011
Wharton, P., & Julian, A. (1996). A Cytological Study of Compatible and Incompatible Interactions between Sorghum Bicolor and *Colletotrichum sublineolum*. *New Phytologist*, *134*(1), 25-34.

Wharton, P., & Schilder, A. (2008). Novel Infection Strategies of *Colletotrichum acutatum* on Ripe Blueberry Fruit. *Plant Pathology*, *57*(1), 122-134.

Wharton, P. S., & Diéguez-Uribeondo, J. (2004). The Biology of *Colletotrichum acutatum*. Anales del jardín botánico de Madrid,

Williams, R. H., Whipps, J. M., & Cooke, R. C. (1998). Role of Soil Mesofauna in Dispersal of *Coniothyrium Minitans*: Mechanisms of Transmission. *Soil Biology and Biochemistry*, *30*(14), 1937-1945.

Wortmann C.S. (1998). Atlas of Common Bean (*Phaseolus vulgaris L.*) Production in Africa. Ciat, Cali, Colombia.

Xie, L., Zhang, J.-z., Wan, Y., & Hu, D.-w. (2010). Identification of *Colletotrichum* Spp. Isolated from Strawberry in Zhejiang Province and Shanghai City, China. *Journal of Zhejiang University Science B*, *11*(1), 61-70.

Yin, L., Cai, M., & Luo, C. (2017). Identification of Anthracnose on Wild Citrus. *Southwest China Journal of Agricultural Sciences*, *30*(3), 590-594.

Zakaria, M., Bailey, J. A., & Pring, R. J. (2000). Infection Processes of *Colletotrichum* Isolates from Forest Trees in the Tropics. *Journal of Tropical Forest Science*, *12*(3), 581-592. http://www.jstor.org/stable/23616281

Zhang, S., Sun, K., Zheng, R., Zeng, H., Wang, S., Chen, R., . . . He, J. (2021). Cancer Incidence and Mortality in China, 2015. *Journal of the National Cancer Center*, *1*(1), 2-11.

Zulfiqar, M., Brlansky, R., & Timmer, L. (1996). Infection of Flower and Vegetative Tissues of Citrus by *Colletotrichum acutatum* and *C. gloeosporioides*. *Mycologia*, *88*(1), 121-128.

Appendix A. Supplementary materials for Chapter 2

A.1 Recipes and procedures for Lactophenol Cotton Blue (LPCB) preparation (in 20mL final volume)

No.	Constituent	Quantity
1	Cotton Blue (Aniline Blue)	0.05 g.
2	Phenol Crystals ($C_6H_5O_4$)	20 g.
3	Glycerol	40 mL.
5	Lactic acid (CH_3CHOH COOH)	20 mL.
6	Distilled water	20 mL.

Preparation procedures

Day 1: Dissolve the cotton blue in distilled water and leave to rest overnight. This eliminates insoluble dye.

Day2: Using protective gloves, add phenol crystals to lactic acid in a glass beaker and stir using a magnetic stirrer until the crystals dissolve. Add glycerol, Filter the Cotton blue and the distilled water into the phenol + glycerol + lactic acid solution, and mix. Store at room temperature for further use.

A.2 Recipes for the agar media tested for growth of *Colletotrichum* species isolates from plant material

Agar type	Recipe
$^1/_2$ strength PDA	15.6 g of potato dextrose agar (PDA; Becton Dickinson & Co, Spark, USA) and 3 g agar in 400 mL of water
Full strength PDA	39 g of PDA in 1 L of water
Malt Extract Agar	Malt extract (Difco™, USA) 20 g and distilled sterilized water 1000 mL.
Water Agar	20.0 g of agar in 1000 mL of distilled water

Antibiotics

+ 5 mg/L of streptomycin and 5 mg/L penicillin (Sigma Aldrich) and penicillin (Sigma Aldrich).

A.3 Mean colony diameter (mm) and spore production (10^4/ml) across five *Colletotrichum* species isolates on four different media tested for main experiment.

Media type	Colony diameter (mm)		Mean	Spore count (x 10^4 spore/ml)		Mean
	20°C	25°C		20°C	25°C	
Half strength PDA	55.3	49.3	52.3	48.3	84.2	66.3
Full strength PDA	36.0	71.7	53.9	51.1	95.5	73.3
Malt Extract Agar	55.2	84.2	69.7	64.5	170.0	117.3
Water Agar	51.8	47.2	49.5	35.1	88.0	61.6

A.4 Mean colony diameter (mm) of five different *Colletotrichum* species isolates incubated at different temperatures under 12 hr light: 12 hr dark or constant dark regimes and assessed after different time intervals.

Isolate	Mean colony diameter (mm) in 12 hr light: dark						
	15 °C						
	48 hr	96 hr	144 hr	192 hr	240 hr	288 hr	336 hr
C.g1 g	2.54 ^{abcde}	4.72 ^{efghIjkl}	8.19 ^{mno}	18.35 ^{uv}	31.37 ^{GHIJ}	45.19 ^{WX}	63.35 ^{g1}
C.g2 f	2.24 ^{abcd}	4.32 d ^{efghIj}	6.59 ^{klmn}	15.35 st	29.37 ^{EFG}	36.19 ^{LM}	50.35 ^{Za1}
C.g3 e	1.94 ^{abc}	3.82 ^{bcdefghi}	6.79 ^{lmn}	16.55 ^{tu}	26.37 ^{ABCD}	32.19 ^{HIJK}	58.35 ^{d1e1}
C.a4 c	1.14 ^{a}	3.32 ^{abcdefgh}	4.49 ^{defghIjk}	13.35 ^{qrs}	23.37 ^{xyz}	40.19 ^{QRS}	44.35 ^{UVW}
C.a5 d	1.64 ^{ab}	2.82 ^{abcdef}	3.99 ^{cdefghi}	12.35 ^{pq}	24.37 ^{yzAB}	36.19 ^{LM}	52.35 ^{a1b1}
Colony diameter (mm) of *Colletotrichum species* in constant dark							
C.g1				1.95 ^{qrst}			2.01 ^{pqrst}
C.g2				2.34 ^{opqrst}			2.6 ^{mnopq}
C.g3				1.77 st			1.93 ^{qrst}
C.a4				1.75 ^{t}			2.03 ^{opqrst}
C.a5				1.96 ^{qrst}			2.32 ^{opqrst}

Mean of 10 replicates for each treatment, values followed with the same letter(s) are not statistically different at p=0.05.

Table A.4 Continued

Isolate	Mean colony diameter (mm)						
	20 ^{0}C						
	48 hr	96 hr	144 hr	192 hr	240 hr	288 hr	336 hr
C.g1	4.82 ^{fghIjkl}	26.4 ^{BCD}	34.13 ^{KL}	36.85 ^{M}	57.78 ^{d1}	72.14 $^{l1\ m1}$	88.14 ^{s1}
C.g2	3.82 ^{bcdefghi}	23.4 ^{xyz}	27.13 ^{CDE}	30.85 ^{GHI}	48.78 ^{YZ}	66.14 $^{h1\ i1}$	66.14 $^{h1\ I1}$
C.g3	4.42 ^{defghIjk}	20.4 ^{vw}	27.85 ^{DEF}	30.13 ^{GH}	37.14 ^{MNOP}	60.14 $^{e1\ f1}$	72.14 $^{l1\ m1}$
C.a4	3.22 ^{Aabcdefg}	13.4 ^{qrs}	22.85 ^{xy}	24.14 ^{xyzA}	43.78 T ^{UVW}	54.14 $^{b1\ c1}$	56.14 $^{c1\ d1}$
C.a5	3.92 ^{cdefghi}	10.4 ^{op}	16.85 ^{tu}	36.13 ^{LM}	46.78 ^{XY}	50.14 $^{Z\ a1}$	68.14 $^{i1\ j1\ k1}$
Colony growth diameter (mm) of *Colletotrichum species* in constant dark							
C.g1				3.08 ^{lmn}			3.62 ^{ijkl}
C.g2				3.5 ^{jkl}			4.01 ^{ghij}
C.g3				4.27 ^{efghi}			4.72 ^{def}
C.a4				2.47 ^{mnopqrs}			3.17 ^{klm}
C.a5				3.84 ^{hijk}			6.62 ^{defg}

Table A.4 Continued

Isolate	Mean colony diameter (mm) in 12 hr light: 12 hr dark						
	25 ^{0}C						
	48 hr	96 hr	144 hr	192 hr	240 hr	288 hr	336 hr
C.g1	18.62 ^{uv}	42.73 T ^{UV}	53.59 ^{b1}	88.77 ^{s1}	*	*	*
C.g2	12.92 ^{qr}	36.73 ^{M}	43.59 T ^{UVW}	78.77 $^{o1\ p1}$	84.77 ^{r1}	88.77 ^{s1}	*
C.g3	15.02 ^{rst}	35.73 ^{LM}	37.59 ^{MNOP}	65.77 ^{h1}	76.77 $^{n1\ o1}$	87.77 ^{s1}	*
C.a4	4.82 ^{fghIjkl}	25.59 ^{zABC}	26.73 ^{CD}	35.77 ^{LM}	44.77 ^{VWX}	68.77 $^{j1\ k1}$	73.77 ^{m1}
C.a5	5.52 ^{hIjkl}	22.03 ^{wx}	32.59 ^{IJK}	39.77 ^{PQR}	41.77 ^{RST}	60.77 ^{f1}	67.77 $^{h1\ i1\ j1}$
Colony diameter (mm) of *Colletotrichum* species in constant dark							
C.g1				5.13 ^{bcd}			5.43 ^{bc}
C.g2				5.57 ^{ab}			6.14 ^{a}
C.g3				4.3 ^{efghi}			4.92 ^{bcde}
C.a4				3.95 ^{ghij}			4.44 ^{defg}
C.a5				4.3 ^{efghi}			4.78 ^{cdef}

Table A.4 Continued

Isolate	Mean colony diameter (mm)						
	30 ^{0}C						
	48 hr	**96 hr**	**144 hr**	**192 hr**	**240 hr**	**288 hr**	**336 hr**
C.g1	6.27 ^{jklmn}	29.48 ^{FG}	32.97 ^{IJK}	39.19 ^{NOP}Q	48.19 ^{YZ}	*	*
C.g2	5.97 l ^{jklm}	23.48 ^{xyz}	29.19 ^{EFG}	29.97 ^{FGH}	42.19 ^{STU}	*	*
C.g3	5.47 ^{ghljkl}	22.97 ^{xy}	26.48 ^{BCD}	33.19 ^{JK}	37.19 ^{MN}	*	*
C.a4	9.47 ^{o}	33.97 ^{KL}	37.48 ^{MNO}	61.19 ^{f1g1}	76.19 ^{n1}	79.77 ^{p1q1}	81.77 ^{q1}
C.a5	8.37 ^{no}	31.97 ^{HIJK}	39.48 ^{OPQ}	49.19 ^{Z}	70.19 ^{k1l1}	86.57 ^{r1s1}	88.77 ^{s1}
Colony diameter (mm) of *Colletotrichum species* in constant dark							
C.g1				2.25 ^{opqrst}			2.73 ^{mno}
C.g2				1.89 ^{rst}			2.36 ^{opqrst}
C.g3				2.07 ^{opqrst}			2.51 ^{mnopqr}
C.a4				4.13 ^{fghij}			4.6 ^{defg}
C.a5				2.38 ^{nopqrst}			2.68 ^{mnop}

A.5 Analysis of variance of the effect of two light regimes on colony diameter of *Colletotrichum* species isolates incubated at different temperatures after 192 hr.

Variate: Colony_diameter_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolate	6	2369	394.8	447.56	<.001
Treatment	3	31200	10400	11789.05	<.001
Illumination	1	116800	116800	132400	<.001
Isolate.Treatment	12	14270	1189	1348.03	<.001
Isolate.Illumination	4	1672	418	473.88	<.001
Treatment.Illumination	3	25820	8607	9756.37	<.001
Isolate.Treatment.Illumination	12	12480	1040	1178.74	<.001
Residual	358	315.8	0.8822		
Total	399	206000			

Fisher's unprotected least significant difference test

Isolate

	Mean	
C.g1	24.11	a
C.g2	20.70	b
C.g3	19.99	c
C.a5	18.74	d
C.a4	18.34	e
C.g2	2.00	f
C.g3	1.50	f

A.6 Effect of incubating different *Colletotrichum* species isolates under 12 hr light: 12 hr dark (Light: Dark) compared with constant dark on the mean colony growth (mm) on malt extract agar assessed after 192 hr.

Isolate	Colony diameter (mm)	
	Light: Dark	Dark
C.g1	45.11a	3.10a
C.g2	37.61b	3.33a
C.g3	36.41c	3.10a
C.a4	33.61e	3.08a
C.a5	34.36d	3.12a

A.7 Analysis of variance of the effect of temperature on colony growth in 12 hr light: 12 hr dark incubated at different temperatures after 192 hr.

Variate: M_Grwth_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
C_Isolate	4	3362.880	840.720	727.27	<.001
Treatment_Temp	3	58103.220	19367.740	16754.10	<.001
C_Isolate.Treatment_Temp	12	26466.240	2205.520	1907.89	<.001
Residual	180	208.080	1.156		
Total	199	88140.420			

Fisher's protected least significant difference test

C_Isolate

	Mean	
C.g1	45.11	a
C.g2	37.61	b
C.g3	36.41	c
C.a5	34.36	d
C.a4	33.61	e

Fisher's protected least significant difference test

Treatment_Temp

	Mean	
25	61.77	a
30	42.39	b
20	30.33	c
15	15.19	d

A.8 Analysis of variance of the effect of temperature on colony growth in continuous dark incubated at different temperatures after 192 hr.

Variate: M_growth_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
C_Isolates	4	1.6615	0.4154	0.69	0.598
Temperatures_T_oc	3	206.3538	68.7846	114.68	<.001
C_Isolates.Temperatures_T_oc	12	70.7397	5.8950	9.83	<.001
Residual	171	102.5670	0.5998		
Total	199	386.6950			

Fisher's unprotected least significant difference test

C_Isolates

	Mean	
C.g2	3.325	a
C.a5	3.120	a
C.g1	3.102	a
C.g3	3.102	a
C.a4	3.075	a

Fisher's unprotected least significant difference test

Temperatures_T_^{o}C

	Mean	
25	4.650	a
20	3.432	b
30	2.544	c
15	1.954	d

A.9 Effect of incubating different *Colletotrichum* species isolates at different temperatures under 12 hr light: 12 hr dark (Light: Dark) on the mean colony diameter (mm) on malt extract agar assessed after 192 hr.

Isolate	Colony growth (mm)				Overall mean
	15°C	20°C	25°C	30°C	
C.g1	18.35 l	34.13 h	88.77 a	39.19 f	45.11 a
C.g2	16.55 m	27.13 j	78.77 b	29.19 i	37.91 b
C.g3	15.35 n	30.13 i	65.77 c	33.19 h	36.11 c
C.a4	13.35 o	24.13 k	35.77 g	61.19 d	33.61 e
C.a5	12.35 p	36.13 g	39.77 f	49.19 e	34.36 d
Mean	15.19 d	30.33 c	61.77 a	42.39 b	

*Means followed by different letter are significantly different at P=0.05.

A.10 Effect of incubating different *Colletotrichum* species isolates at different temperatures under constant dark on the mean colony diameter (mm) on malt extract agar assessed after 192 hr.

Isolate	Colony growth (mm)				Overall mean
	15°C	20°C	25°C	30°C	
C.g1	1.95 fg	3.08 de	5.13 a	2.25 fg	3.10 a
C.g2	2.34 fg	3.5 cd	5.57 a	1.89 fg	3.33 a
C.g3	1.77 g	4.27 b	4.3 b	2.07 fg	3.10 a
C.a4	1.75 g	2.47 ef	3.95 bc	4.13 bc	3.08 a
C.a5	1.96 fg	3.84 bc	4.3 b	2.38 fg	3.12 a
Overall mean	1.95 d	3.43 b	4.65 a	2.54 c	

*Means followed by different letter are significantly different at P=0.05.

A.11 Colony diameter at different temperatures in a 12:12 hr light dark regime and spore production (x10^4/mL) of *Colletotrichum* species at different temperatures in constant dark.

Isolate	Mean colony diameter (mm)							
	15^{0}C		20^{0}C		25^{0}C		30^{0}C	
	192 hr	336 hr	192 hr	336 hr	192 hr	336 hr	192 hr	336 hr
C.g1 b	4.544	3.66	13.36	5.62	13.48	10.35	5.58	4.63
C.g2 2	3.6	2.45	7.09	7.14	21.13	16.77	48.69	27.26
C.g3 ab	0.386	2	6.59	3.24	14.1	7.27	6.38	6.75
C.a4 a	*	0.371	0.9	0.957	8.144	5.64	4.757	11.93
C.a5 b	2.82	1.76	8.01	5.74	10.39	6.95	11.13	13.26
Spore production (nx10^4/mL) of *Colletotrichum* species in constant dark								
C.g1 b	2.95	2.95	2.95	2.95	31.15	20.45	11.8	4.75
C.g2 a	0	0.7	2.25	1.35	16.8	4.25	2.8	0.45
C.g3 a	0	0.6	0.25	0.35	13.55	0.5	2.95	0
C.a4 c	0.2	3.75	36.95	21.5	29.55	44.25	34.2	12.45
C.a5 a	0.4	0.25	1.9	2.65	6.7	2.7	0.45	0.8

A.12 Analysis of variance of the effect of two light regimes on spore production (10^4 spore/mL) of *Colletotrichum* species isolates incubated at different temperatures for 192 hr.

Variate: SPORE_SCOUNT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolate	4	176707	44177	9.52	<.001
Illumination	1	373504	373504	80.51	<.001
Isolate.Illumination	4	198249	49562	10.68	<.001
Residual	390	1809388	4639		
Total	399	2557849			

Fisher's unprotected least significant difference test
Isolate

	Mean	
C.g1	74.22	a
C.a5	42.18	b
C.a4	40.61	b
C.g3	25.11	bc
C.g2	11.38	c

A.13 Effect of incubating different Colletotrichum species isolates under 12 hr light: 12 hr dark (Light: Dark) compared with constant dark on the mean spore production (10^4 spore/mL) on malt extract agar assessed for 192 hr.

Isolate	Mean spore production (10^4 spore/mL)	
	Light: Dark	Dark
C.g1	146.5 a	2.0 a
C.g2	19.1 c	3.6 a
C.g3	47.9 bc	2.3 a
C.a4	58.6 b	22.9 b
C.a5	74.1 b	10.2 c

*Means followed by different letter are significantly different at P=0.05.

A.14 Analysis of variance of the effect of temperature on spore production (10^4 spore/mL) in 12 hr light: 12 hr dark incubated at different temperatures for 192 hr.

Variate: Spore_count_10_4_ml_light

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	475967.	158656.	52.35	<.001
Isolate	4	362771.	90693.	29.93	<.001
Treatment.Isolate	12	775051.	64588.	21.31	<.001
Residual	180	545516.	3031.		
Total 199	2159305.				

Bonferroni test

Treatment

	Mean	
15	12.54	a
20	49.20	b
30	69.22	b
25	146.07	c

Bonferroni test

Isolate

	Mean	
C.g1	146.47	a
C.a5	74.14	b
C.a4	58.66	b
C.g3	47.90	bc
C.g2	19.11	c

A.15 Analysis of variance of the effect of temperature on spore production (10^4 spore/mL) in constant dark incubated at different temperatures for 192 hr.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolate	4	25123.87	6280.97	114.19	<.001
Treatment	3	13168.36	4389.45	79.8	<.001
Isolate.Treatment	12	9396.65	783.05	14.24	<.001
Residual	360	19802.13	55.01		
Total	399	74874.11	187.65		

Bonferroni test

Isolate

	Mean	
Ca5	10.219	b
C.g3	2.275	a
C.g2	3.575	a
C.g1	1.981	a
C.a4	22.856	c

Bonferroni test

Treatment

	Mean	
15	0.925	a
30	7.065	b
20	7.745	b
25	16.990	c

A.16 Effect of incubating different *Colletotrichum* species isolates at different temperatures under 12 hr light: 12 hr dark (Light: Dark) on mean spore production (10^4/mL) on malt extract agar assessed for 192 hr.

Isolate	Mean spore production (1x10^4/spore/mL)				Mean
	15°C	20°C	25°C	30°C	
C.g1	28.6 m	93.5 i	424.8 a	39 gh	146.5 a
C.g2	0 q	0.6 l	56.1 d	19.8 h	19.1 c
C.g3	1.9 p	46.2 jk	98.7 b	44.8 gh	47.9 bc
C.a4	12.5 no	49.6 jk	78 c	94.4 f	58.6 b
C.a5	19.8 n	56.1 jk	72.7 c	147.9 e	74.1 b
Mean	12.56 a	49.2 b	146.1 c	69.2 b	

*Means within a column followed by different letter are significantly different at p=0.05.

A.17 Effect of incubating different *Colletotrichum* species isolates at different temperatures under continuous Dark) on mean spore production (10^4/mL) on malt extract agar assessed after 192 hr.

Isolate	Mean spore production (1x10^4/spore/mL)				Mean
	15°C	20°C	25°C	30°C	
C.g1	0.3 j	2.3 e	4.7 bc	0.6 hi	2.0 a
C.g2	0.4 j	1.8 e	10.5 b	1.6 hi	3.6 a
C.g3	0.3 j	0.3 e	7 b	1.5 hi	2.3 a
C.a4	2 jk	29.2 c	36.9 a	23.3 f	22.9 b
C.a5	1.7 jk	5.1 d	25.8 a	8.3 g	10.2 c
Mean	0.9 a	7.8 b	17.0 c	7.1 b	

*Means within a column followed by different letter are significantly different at p=0.05.

A.18 Correlation Coefficient (r) for the relationship between colony growth (mm) and spore production for the five *Colletotrichum* species isolates incubated under 12:12 light: dark regime at 25°C and assessed after 192 hr.

Isolate	Correlation coefficient (r)	P value
C.g1	0.68	0.03
C.g2	0.19	0.60
C.g3	0.11	0.77
C.g4	0.42	0.23
C.g5	0.16	0.65

a. Isolate C.g1

Regression Statistics	
Multiple R	0.68
R Square	0.46
Adjusted R Square	0.39
Standard Error	1.56
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	16.67	16.67	6.84	0.03
Residual	8.00	19.50	2.44		
Total	9.00	36.18			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-109.75	47.43	-2.31	0.05	-219.12	-0.39	-219.12	-0.39
Mean colony growth (mm)	1.40	0.53	2.62	0.03	0.17	2.63	0.17	2.63

b. Isolate C.g2

Regression Statistics	
Multiple R	0.19
R Square	0.04
Adjusted R Square	-0.09
Standard Error	10.54
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	32.57	32.57	0.29	0.60
Residual	8.00	888.61	111.08		
Total	9.00	921.18			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-132.69	284.08	-0.47	0.65	-787.79	522.41	-787.79	522.41
Mean colony growth (mm)	1.95	3.61	0.54	0.60	-6.36	10.27	-6.36	10.27

c. Isolate C.g3

Regression Statistics	
Multiple R	0.11
R Square	0.01
Adjusted R Square	-0.11
Standard Error	6.59
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	3.97	3.97	0.09	0.77
Residual	8.00	347.55	43.44		
Total	9.00	351.52			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-30.72	148.35	-0.21	0.84	-372.81	311.37	-372.81	311.37
Mean colony growth (mm)	0.68	2.26	0.30	0.77	-4.52	5.88	-4.52	5.88

d. Isolate C.a4

Regression Statistics	
Multiple R	0.42
R Square	0.18
Adjusted R Square	0.07
Standard Error	2.27
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	8.74	8.74	1.70	0.23
Residual	8.00	41.18	5.15		
Total	9.00	49.92			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-28.18	27.78	-1.01	0.34	-92.24	35.87	-92.24	35.87
Mean colony growth (mm)	1.01	0.78	1.30	0.23	-0.78	2.80	-0.78	2.80

e. Isolate C.a5

Regression Statistics	
Multiple R	0.16
R Square	0.03
Adjusted R Square	-0.10
Standard Error	2.03
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.89	0.89	0.22	0.65
Residual	8.00	32.88	4.11		
Total	9.00	33.77			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-2.45	27.60	-0.09	0.93	-66.08	61.19	-66.08	61.19
Mean colony growth (mm)	0.32	0.69	0.47	0.65	-1.28	1.92	-1.28	1.92

A.19 Correlation Coefficient (r) for the relationship between colony growth (mm) and spore production for the five *Colletotrichum* species isolates incubated under 12:12 light: dark regime at 25°C and assessed after 336 hr.

Isolate	**Correlation coefficient (r)**	**P value**
C.g1	0.53	0.0003
C.g2	0.36	0.019
C.g3	0.36	0.019
C.g4	0.53	0.0004
C.g5	0.57	0.0001

A.20 Correlation Coefficient (r) for the relationship between colony growth (mm) and spore production for the five *Colletotrichum* species isolates incubated in constant dark at 25°C and assessed after 192 hr.

Isolate	Correlation coefficient (r)	P value
C.g1	0.02	0.95
C.g2	0.13	0.73
C.g3	1.44	0.99
C.g4	0.37	0.29
C.g5	0.53	0.12

a) Isolate C.g1

Regression Statistics	
Multiple R	0.02
R Square	0.00
Adjusted R Square	-0.12
Standard Error	0.27
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	0.00	0.95
Residual	8.00	0.58	0.07		
Total	9.00	0.58			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.13	2.62	0.43	0.68	-4.91	7.16	-4.91	7.16
Mean colony growth (mm)	-0.04	0.73	-0.06	0.95	-1.73	1.64	-1.73	1.64

b) Isolate C.g2

Regression Statistics	
Multiple R	0.13
R Square	0.02
Adjusted R Square	-0.11
Standard Error	0.57
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.04	0.04	0.13	0.73
Residual	8.00	2.57	0.32		
Total	9.00	2.61			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.01	1.06	1.89	0.10	-0.44	4.46	-0.44	4.46
Mean colony growth (mm)	0.06	0.17	0.36	0.73	-0.34	0.46	-0.34	0.46

c) Isolate C.g3

Regression Statistics	
Multiple R	1.44
R Square	2.07
Adjusted R Square	-1.25
Standard Error	7.32
Observations	1.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	8.88	8.88	1.66	0.99
Residual	8.00	4.29	5.36		
Total	9.00	4.29			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.35	15.64	0.09	0.93	-34.71	37.41	-34.71	37.41
Mean colony growth (mm)	0.00	2.36	0.00	1.00	-5.45	5.45	-5.45	5.45

d) Isolate C.a4

Regression Statistics	
Multiple R	0.37
R Square	0.14
Adjusted R Square	0.03
Standard Error	0.74
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.68	0.68	1.26	0.29
Residual	8.00	4.34	0.54		
Total	9.00	5.03			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-2.84	6.27	-0.45	0.66	-17.29	11.61	-17.29	11.61
Mean colony growth (mm)	1.85	1.65	1.12	0.29	-1.95	5.65	-1.95	5.65

e) Isolate C.a5

Regression Statistics	
Multiple R	0.53
R Square	0.28
Adjusted R Square	0.19
Standard Error	0.59
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	1.07	1.07	3.09	0.12
Residual	8.00	2.76	0.34		
Total	9.00	3.82			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-5.13	5.05	-1.02	0.34	-16.77	6.51	-16.77	6.51
Mean colony growth (mm)	3.25	1.85	1.76	0.12	-1.01	7.51	-1.01	7.51

A.21 Consensus sequences of the *ITS* region amplified using primers ITS1 and ITS4 for *Colletotrichum* species isolates recovered from symptomatic avocado and apple fruit.

Isolate C.g1
AUCAUUACUGAGUUUACGCUCUACAACCCUUUGUGAACATACCTATAACTGTTGCTTCGGCGGGCAGGGTCTC
CGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACG
TTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCC
AGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGCTG
ACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGAT
CCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACT
TAAGCATATCAAT

Isolate C.g2
CGGAGGGATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGCAGGG
TCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACG
ACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
GCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGG
CTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGG
GATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTG
AACTTAAGCATATCAA

Isolate C.g3
UACUGAGUUUACGCUCUACAACCCUUUGUGAACAUACCUATAACTGTTGCTTCGGCGGGCAGGGTCTCCGTGA
CCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTT
CTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATT
CTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGCTGACGTAG
GCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAG
GGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA

Isolate C.a4
CCUGCGGAGGGAUCAUUACUGAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGGCG
GGCAGGGGAAGCCUCUCGCGGGCCUCCCCUCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAG
GAAACCAAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCT
TGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCG
CTTGGTTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTA
GTAACTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGG
ATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAT

Isolate C.a5
GCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCCC
TCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACACGACGTCTCTT
CTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGA

AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATT
CTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTGG
GCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTGGGATCCGGAG
GGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGC

Appendix B. Supplementary materials for Chapter 3

B.1 Potting mix recipes used in the recovery of CFU from plant material inoculated with suspension of *Colletotrichum* species Isolates.

Material
80% composted bark
20% pumice
Grade 1-3mm
Osmocote™ exact
16-3.9-10 (NPK) 3-4 month release 3g/L
Horticultural lime 1g/L
Hydraflo™ (wetting agent) 1g/L

B.2 Symptom rating scale used to assess disease severity (total infection) on whole *Phaseolus vulgaris* plants.

Plant A	Characteristics (% infection)	Score (0-5)	Plant B	Characteristics (% infection	Score
	5=81-100% leaves infected or plant is dead **Features** - Gross chlorosis - Retarded growth - Plant progressively dyeing	5		4=61-80% leaves of plant infected. **Features** - Progressive chlorosis - Dying of lower leaves	4
Plant C			**Plant D**		
	3=41-60% leaves of plant infected **Features** - Leaf distortion - Brown lesions on leaves are prominent - Yellowing is beginning to become visible on lower leaves	3		2=21-40% leaves of plant infected **Features** - Distortion of younger leaves - Brown lesion beginning to show up - Mild yellowing of leaves	2
Plant E			**Plant F**		
	1=1-20% leaves of plant infected. **Features** - Brown spots are visible on lower leaves	1		0= No infection/lesion **Features.** - Plant is healthy.	0

B.3 Analysis of variance (ANOVA) of the effect of different *Colletotrichum* species isolates and inoculum concentration (doses) on disease incidence on leaves (arcsine transformed data).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Doses	3	7.5717	2.5239	14.55	<.001
Isolate	3	2.0046	0.6682	3.85	0.012
Treatment	5	0.8222	0.1644	0.95	0.454
Doses.Isolate	3	1.1236	0.3745	2.16	0.097
Residual	105	18.2187	0.1735		
Total	119	29.7408			

Bonferroni test

Doses

	Mean	
4	0.3148	a
1	0.7164	b
2	0.8085	b
3	1.0041	b

B.4 Analysis of variance (ANOVA) of the effect of different *Colletotrichum* species isolates and inoculum concentration (doses) on disease incidence on pods (arcsine transformed data).

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Doses	3	4.4555	1.4852	7.41	<.001
Isolate	3	0.5676	0.1892	0.94	0.422
Treament	5	0.3531	0.0706	0.35	0.88
Doses.Isolate	3	0.4244	0.1415	0.71	0.551
Residual	105	21.0478	0.2005		
Total	119	26.8484			

Bonferroni test

Doses

	Mean	
4	0.1113	a
1	0.5026	b
2	0.5393	b
3	0.6035	b

B.5 Analysis of variance (ANOVA) of the effect of different *Colletotrichum* species isolates and inoculum concentration (doses) on overall disease score on *P. vulgaris* plants (arcsine transformed data).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Doses	3	89.425	29.8083	34.43	<.001
Isolate	3	4.8222	1.6074	1.86	0.141
Treatment	5	1.2667	0.2533	0.29	0.916
Doses.Isolate	3	5.9111	1.9704	2.28	0.084
Residual	105	90.9	0.8657		
Total	119	192.325			

Bonferroni test

Doses

	Mean	
4	1.467	a
1	3.167	b
2	3.367	b
3	3.700	b

B.6 Consensus sequence results for the ITS region amplified using primers ITS1 and ITS4 for representative isolates recovered from infected P. vulgaris.

BLPC.a2
TAACCGTTGCTTCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCCCACCACGGGGACG
GGGCGCCCCGCSGGAGGAAACCAAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACT
TTTUAACAACGGATCTCTTUGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTC
ATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTC
CCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCKCWUCTSGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAAT
TCTTTACAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACT

BLPC.a4
TTACTGAGTTACCGCTCTATAACCCTTTGTGAACGTACCTAACCGTTGCTTCGGCGGGCAGGGGAAGCCTCTCGCG
GGCCTCCCCTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACACG
ACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTC
GCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGG
CCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTGG
GATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGAATACCCGCTG
AAC

BLPC.a4
AATCCGTTGCTTCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCCCACCACGGGGACG
GGGCGCCCCGCSGGAGGAAACCAAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACT
TTTUAACAACGGATCTCTTUGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTC
ATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTC
CCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCKCWUCTSGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAAT
TCTTTACAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGTTC

BLPC.g1
CTGTTGCTTCGGCGGGCAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGG
ATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCT
TGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTG
CTTGGTGTTGGGGCCCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTA
GTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGG
ATCAGGTAGGAATACCCGCTGAACTTTAAGCATAT

BLPC.g2
CGGAGGGAUCAUUACUGAGUUUACGCUCUACAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGCAG
GGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTA
ACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCT

ACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCA
CTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCA

BLPC.g3
CGGAGGGAUCAUUACUGAGUUUACGCUCUACAACCCUUUGUGAACAUACCUAUAACUGUUGCUUCGGCGGG
CAGGGUCUCCGUGACCCUCCCGGCCUCCCGCCCCCGGGCGGGUCGGCGCCCGCCGGAGGAUAACCAAACUCTG
ATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA
TTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGG
CCCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCT
CGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAAT
ACCCGCTGAAC

B.7 BLAST result of comparison of recovered isolates from bean leaves and pods with the mother isolates from infected fruits.

Mother isolate	Source	Recovered Isolates	Source	Identity	Query cover	% identity	Accession number	Length (bp)
C.g1	Avocado	BLPC.g1	Bean pods	*Colletotrichum gloeosporioides* strain	100%	100	MF495411.1	599
		BLPC.g2	Bean leaves	*Colletotrichum gloeosporioides* isolate	100%	100	MT083978.1	593
C.g3	Avocado	BLPC.g3	Leaves	*Colletotrichum kahawae* isolate	100%	100	MT133276.1	588
C.a4	Apple	BLPC.a2	Leaves	*Colletotrichum fioriniae* isolate	100%	100	MT133292.1	595
		BLPC.a5	Leaves	*Colletotrichum fioriniae* isolate	100%	100	MT133290.1	596

Appendix C. Supplementary material for Chapter 4

C.1 Moisture maintenance in the potting mix: soil mixture during the experiment

Replicate	**Initial**			**Moisture adjustment (Water in mL)**					
	Wet weight (WW) /g	Dry weight (DW) /g	%WHC	Day 1 WW	%WHC	Day 2 WW	%WHC	Day 3 WW	%WHC
R1T1	20	13	53.85	0.80	60.00				
R2T1	19	12	58.33	0.20	60.00				
R3T1	21	13	61.54	0.00	61.54				
R4T1	17	13	30.77	3.80	60.00				
R5T1	19	13	46.15	1.80	60.00				
R6T1	15	12	25.00	4.20	60.00				
R7T1	17	12	41.67	2.20	60.00				
R8T1	17	14	21.43	5.40	60.00				
R9T1	20	14	42.86	2.40	60.00				
R10T1	18	12	50.00	1.20	60.00				
R1T2	19	13	46.15	1.80	60.00				
R2T2	19	14	35.71	3.40	60.00				
R3T2	20	13	53.85	0.80	60.00				
R4T2	20	13	53.85	0.80	60.00				
R5T2	21	14	50.00	1.40	60.00				
R6T2	20	14	42.86	2.40	60.00				
R7T2	21	13	61.54	0.00	61.54				
R8T2	21	13	61.54	0.00	61.54				
R9T2	20	13	53.85	0.80	60.00				
R10T2	20	13	53.85	0.80	60.00				
R1T3	19	12	58.33	0.20	60.00				
R2T3	17	12	41.67	2.20	60.00				
R3T3	19	12	58.33	0.20	60.00				
R4T3	17	13	30.77	3.80	60.00				
R5T3	21	13	61.54	0.00	61.54				
R6T3	20	13	53.85	0.80	60.00				
R7T3	21	13	61.54	0.00	61.54				
R8T3	21	13	61.54	0.00	61.54				
R9T3	21	13	61.54	0.00	61.54				
R10T3	21	13.5	55.56	0.60	60.00				
R1T4	21	13	61.54	0.00	61.54				
R2T4	20	13	53.85	0.80	60.00				

R3T4	18	12	50.00	1.20	60.00
R4T4	17	11	54.55	0.60	60.00
R5T4	17	11	54.55	0.60	60.00
R6T4	21	13	61.54	0.00	61.54
R7T4	20	13	53.85	0.80	60.00
R8T4	20	13	53.85	0.80	60
R9T4	19	12	58.33	0.20	60.00
R10T4	21	13	61.54	0.00	61.54
R1T5	17	13	30.77	3.80	60.00
R2T5	19	13	46.15	1.80	60.00
R3T5	15	12	25.00	4.20	60.00
R4T5	18	12	50.00	1.20	60.00
R5T5	17	12	41.67	2.20	60.00
R6T5	16	11	45.45	1.60	60.00
R7T5	18	12	50.00	1.20	60.00
R8T5	19	13	46.15	1.80	60.00
R9T5	19	14	35.71	3.40	60.00
R10T5	20	13	53.85	0.80	60.00
R1T6	20	13	53.85	0.80	60.00
R2T6	21	14	50.00	1.40	60.00
R3T6	20	14	42.86	2.40	60.00
R4T6	21	14	50.00	1.40	60.00
R5T6	21	13	61.54	0.00	61.54
R6T6	21	13	61.54	0.00	61.54
R7T6	19	12	58.33	0.20	60.00
R8T6	16	10	60.00	0.00	60.00
R9T6	17	12	41.67	2.20	60.00
R10T6	19	12	58.33	0.20	60.00
R1T7	17	13	30.77	3.80	60.00
R2T7	21	13	61.54	0.00	61.54
R3T7	20	13	53.85	0.80	60.00
R4T7	21	13	61.54	0.00	61.54
R5T7	21	13	61.54	0.00	61.54
R6T7	21	14	50.00	1.40	60.00
R7T7	17	11	54.55	0.60	60.00
R8T7	16	11	45.45	1.60	60.00
R9T7	19	12	58.33	0.20	60.00
R10T7	20	13	53.85	0.80	60.00
R1T8	19	13	46.15	1.80	60.00
R2T8	19	13	46.15	1.80	60.00
R3T8	21	13	61.54	0.00	61.54

R4T8	17	12	41.67	2.20	60.00
R5T8	18	12	50.00	1.20	60.00
R6T8	17	11	54.55	0.60	60.00
R7T8	19	13	46.15	1.80	60.00
R8T8	21	13	61.54	0.00	61.54
R9T8	17	11	54.55	0.60	60.00
R10T8	21	13	61.54	0.00	61.54

C.2 Recipes for the agar media tested for isolation of *Colletotrichum* species recovered from plant material

Agar type	**Recipe**
$^1/_5$ strength PDA	3.12 g potato dextrose agar (PDA; Becton Dickinson & Co, Spark, USA) and 4.8 g of agar in 400 mL of water + 10 mg/L each of streptomycin (Sigma Aldrich) and penicillin (Sigma Aldrich).
$^1/_2$ strength PDA	7.8 g of PDA and 15.6 g agar in 400 mL of water + 10 mg/L of streptomycin and penicillin.
Full strength PDA	39 g of PDA in 1 L of water + 10 mg/L of streptomycin and penicillin
Prune agar	100 mL prune, 5.0 g sucrose, 1.0 yeast extract, 30 g agar in 1 L of water + 10 mg/L of streptomycin and penicillin (Difco™)
V8 juice agar	200 mL Campbells V8 juice 0.75 g of calcium carbonate and 15 g agar in 800 mL of water + 10 mg/L of streptomycin and penicillin

C.3 Analysis of variance of the effects of burying and blending of plant material on CFU after inoculating with suspension of *C. acutatum* isolate.

Variate: CFU_g					
Source of variation	d.f.	S.S.	M.S.	v.r.	F pr.
Treatment	7	2.880E+11	4.114E+10	59.30	<.001
Lin	1	2.051E+11	2.051E+11	295.70	<.001
Deviations	6	8.286E+10	1.381E+10	19.90	<.001
Residual	72	4.995E+10	6.938E+08		
Total	79	3.380E+11			

Fisher's unprotected least significant difference test

Treatment	**Mean CFU/g plant tissue**	
1	167000	a
3	136333	b
2	67333	c
4	54333	c
8	8933	d
5	8333	d
7	8333	d
6	0	d

C.4 Analysis of variance of the effects of burying and blending of plant material on CFU after inoculating with suspension of *C. gloeosporioides* isolate.

Variate: CFU_g					
Source of variation	d.f.	S.S.	M.S.	v.r.	F pr.
Treatment	7	1.227E+11	1.753E+10	33.14	<.001
Lin	1	8.720E+10	8.720E+10	164.80	<.001
Deviations	6	3.553E+10	5.922E+09	11.19	<.001
Residual	72	3.810E+10	5.291E+08		
Total	79	1.608E+11			

Fisher's unprotected least significant difference test

Treatment	**Mean CFU/g plant tissue**	
2	97667	a
1	94600	a
3	22333	b
4	13000	bc
5	4833	bc
8	1666	c
6	667	c
7	333	c

C.5 Analysis of variance of the mean colony growth rate of *Colletotrichum* species isolates incubated at 25°C in 12: 12hr photoperiod.

SUMMARY

Isolate groups	*Count*	*Sum*	*Average*	*Variance*
Isolate groups	4.00	10.00	2.50	1.67
Colony growth rate (mm/day)	160.00	964.70	6.03	2.74

ANOVA

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Between isolate groups	1	48.61	48.61	17.89	<.001	3.90
Within isolate groups	162	440.29	2.72			
Total	163	488.90				

Bonferroni test

Isolate group

	Mean	
1	6.62	a
2	5.93	b
3	5.81	b
4	4.50	c

C.6 Analysis of variance of the mean conidial length of *Colletotrichum* species isolates incubated at 25°C in 12: 12hr photoperiod.

SUMMARY

Isolate groups	*Count*	*Sum*	*Average*	*Variance*
Isolate groups	4	10	2.5	1.67
Colony growth rate (mm/day)	160	2,601.20	16.26	11.38

ANOVA

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Between isolate groups	1	738.61	738.61	65.95	<.001	3.90
Within isolate groups	162	1,814.29	11.20			
Total	163	2,552.90				

Bonferroni test

Isolate group

	Mean	
1	16.51	c
2	16.22	c
3	14.00	b
4	24.14	a

C.7 Analysis of variance of the mean conidial width of *Colletotrichum* species isolates incubated at 25°C in 12: 12hr photoperiod.

SUMMARY

Isolate groups	*Count*	*Sum*	*Average*	*Variance*
Isolate groups	4	10	2.5	1.67
Colony growth rate (mm/day)	160	2,601.20	16.26	11.38

ANOVA

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Between isolate groups	1	738.61	738.61	65.95	<.001	3.90
Within isolate groups	162	1,814.29	11.20			
Total	163	2,552.90				

Bonferroni test

Isolate group

	Mean	
1	5.54	c
2	5.51	c
3	5.22	b
4	3.74	a

C.8 Consensus sequence results of isolates recovered from plant debris.

SCa and SCt isolates

Sca1
GCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACACGACGTCTCTTCTGAGTG
GCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATTCTGGCG
AGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTGGGCCCTT
AAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTGGGATCCGGAGGGACTC
TTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAAC

SCa2
UGCGGAGGGAUCAUUACUGAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGGCGGG
CAGGGGAAGCCUCUCGCGGGCCUCCCCUCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCSGGAGGA
AACCAAACTCTATTTACACGACGTCTCTTCTGAGUGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTG
GTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCT
TGGTTTTGGGGCCCCACGGCCGACGKGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGT
AACTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACA

SCa3
UAAGUUCAGCGGGUAUUCCUACCUGAUCCGAGGUCAACCUGUAAAGAATTTGGGGGTTTAACGGCAAGAGTC
CCTCCGGATCCCAGTGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCCACTACCTTTAAGGG
CCCACGTCGGCCGTGGGGCCCCAAAACCAAGCGGTGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCTCGC
CAGAATGCTGGCGAGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGC
ATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACTCA
GAAGAGACGTCGTGTAAATAGAGTTTGGTTTCCTCCGGCGGGCGCCCCGTCCCCGTGGTGGGGGCCGGCGCCGG
GAGGGGAGGCCCGCGAGAGGCTTCCCCTGCCCGCCGAAGCAACGGTTAGGTACGTTCACAAAGGGTTATAGAGC
GGTAACTCAGTAATGATCCCTCCGCA

SCa4
GGAGGGAUCAUUACUGAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGGCGGGCAG
GGGAAGCCUCUCGCGGGCCUCCCCUCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAAC
CAAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTT
CTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTG
GTTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAA
CTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCA
GGTAGGAATACCCGCTGAACTTAA

Sca5
GAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGGCGGGCAGGGGAAGCCUCUCGCG
GGCCUCCCCUCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACAC
GACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCT
CGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACG
GCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTG

GGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGAATACCCGCT
GAAC

Sca6
CCTGCGGAGGGATCATTACTGAGTTACCGCTCTATAACCCTTTGTGAACGTACCTAACCGTTGCTTCGGCGGGCAG
GGGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACC
AAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTC
TGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGG
TTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAAC
TAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAG
GTAGGAATACCCGCTGAACTTAAGCATAT

Ca7
TGCGGAGGGATCATTACTGAGTTACCGCTCTATAACCCTTTGTGAACGTACCTAACCGTTGCTTCGGCGGGCAGGG
GAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAA
ACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTT
TGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAA
CGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTA
GGAATACCCGCTGAACTTAAG

SCa8
AGGGUGAACCUGCGGAGGGAUCAUUACUGAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUG
CTTCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCC
GCCGGAGGAAACCAAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
TCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTC
AAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCC
TTTGCGTAGTAACTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTT
GACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGC

SCa9
AACCUGCGGAGGGAUCAUUACUGAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGG
CGGGCAGGGGAAGCCUCUCGCGGGCCUCCCCUCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGG
AGGAAACCAAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCT
CTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
AATCTTTGAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCAC
CGCTTGGTTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCG
TAGTAACTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTC
GGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATA

SCa10
GGUGAACCUGCGGGAGGGAUCAUUACUGAGUUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUG
CUUCGGCGGGCAGGGGAAGCCCUCUCGCGGGCCUCCCCUCCCGGCGCCGGCCCCCACCACGGGGACGGGGCG
CCCGCCGGAGGAAACCAAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAAC
AACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT
GAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAAC

CCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGC
CTCCTTTGCGTAGTAACTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAG
GTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAG

SCa11
CGGAGGGAUCAUUACUGAGUUACCGCUCUAUAACCCUUUGUGAACGTACCTAACCGTTGCTTCGGCGGGCAGG
GGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCA
AACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCT
GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA
ACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGT
TTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACT
AACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGG
TAGGAATACCCGCTGAACTT

Sca12
AUUGAUAUGCUUAAGUUCAGCGGGUAUUCCUACCUGAUCCGAGGUCAACCUGUAAAGAAUUUGGGGGUUU
AACGGCAAGAGTCCCTCCGGATCCCAGTGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
ACTACCTTTAAGGGCCCACGTCGGCCGTGGGGCCCCAAAACCAAGCGGTGCTTGAGGGTTGAAATGACGCTCGAA
CAGGCATGCTCGCCAGAATGCTGGCGAGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA
CATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTG
CTTGTGCCACTCAGAAGAGACGTCGTGTAAATAGAGTTTGGTTTCCTCCGGCGGGCGCCCCGTCCCCGTGGTGGG
GGCCGGCGCCGGGAGGGGAGGCCCGCGAGAGGCTTCCCCTGCCCGCCGAAGCAACGGTTAGGTACGTTCACAAA
GGGTTATAGAGCGGTAACTCAGTAATGATCCCTCCG

Sca13
CCGCUCUAUAACCCUUUGUGAACGUACCUAACCGTTGCTTCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCC
CTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACACGACGTCTCT
TCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCAT
TCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTG
GGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTGGGATCCGGA
GGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGGTAGGAATACCCGCTGAACTTAA
GCATATCAATAAGGCGGAG

Sct14
GAGGGAUCAUUUACUGAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGGCGGGCAG
GGGAAGCCUCUCGCGGGCCUCCCCTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAAC
CAAACTCTATTTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTT
CTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTG
GTTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAA
CTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCA
GGTAGGAATACCCGCTGAACTTA

Sct15
AUUACUGAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGGCGGGCAGGGGAAGCCU
CUCGCGGGCCUCCCCUCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTAT
TTACACGACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGA

TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACAT
TGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGC
CCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTC
GCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGAATA
CCCGCTGAACTTAAGCATA

Sct16
CCGCUCUAUAACCCUUUGUGAACGUACCUAACCGTTGCTTCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCC
CTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACACGACGTCTCT
TCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCAT
TCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTG
GGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTGGGATCCGGA
GGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGGTAGGAATACCCGCTGAACTTAA
GCATATCAATAAGGCGGAG

SCg and SCf Isolates

SCg1
ACTGTTGCTTCGGCGGGCAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAG
GATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTC
TTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
ATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCT
GCTTGGTGTTGGGGCCCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGT
AGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCG
GATCAGGTAGGAATACCCGCTGAACTTAAGCATAT

SCg2
GGGAUCAUUACUGAGUUUACGCUCUACAACCCUUUGUGAACAUACCUAUAACUGUUGCUUCGGCGGGCAGG
GUCUCCGUGACCCUCCCGGCCUCCCGCCCCCGGGCGGGUCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTA
ACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCGCCAGCATTCTGGCGGGCATGCCUGUUCGAGCGUCAUUUCAACCCUCAAGCUCUGCUUGGUGUUG

SCg3
GGGATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGCAGGGTCTCC
GTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGT
TTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGKTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAG
CATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGCTGAC
GTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCC
GGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTA
AGCATATCAATAA

Scg4
CCGCUCUAUAACCCUUUGUGAACGUACCUAACCGTTGCTTCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCC
CTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACACGACGTCTCT
TCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCAT

TCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCCGACGTG
GGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTGGGATCCGGA
GGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGGTAGGAATACCCGCTGAACTTAA
GCATATCAATAAGGCGGAG

Scg5
UGUGAACAUACCACUUGUUGCCUCCGGCGSAUCRGCCCSCWCCACGGKRAAACGGGRCGGCCCGCCRGAGGA
MMCCUAAACTCTRTTTMYAYGAYGTMWCTTCTGAGTRRMACMAKCAAATAAWTCAAAACTTTYAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCRAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAATCTTTGAACGCACATTGCGCYCGCCAGYATTCTGGCGRGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCA
CMGCTTGGTKTTGGGRCYCSACGKYMRWUCGYGKKCCCTYAAAKKKAKTGGCGGWCCCTCCCGGAGCCTCCTTTG
CGTAGTAACTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACC
TCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAA

SCg6
UGCGGAGGGAUCAUUACUGAGUUUACGCUCUACAACCCCUUUGUGAACATACCTATAACTGTTGCTTCGGCCG
GGCAGGGTCTCCGTGACCCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCT
GATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATC
GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGG
GCCCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTC
TCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAA
TACCCGCTGA

SCg7
GGGAUCAUUACUGAGUUUACGCUCUACAACCCUUUGUGAACAUACCUAUAACUGUUGCUUCGGCGGGCAGG
GUCUCCGUGACCCUCCCGGCCUCCCGCCCCCGGGCGGGUCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTA
ACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCGCCAGCATTCTGGCGGGCATGCCUGUUCGAGCGUCAUUUCAACCCUCAAGCUCUGCUUGGUGUUG

Scg8
AUUACUGAGUUUACGCUCUACAACCCUUUGUGAACAUACCUAUAACUGTTGCTTCGGCGGGCAGGGTCTCCG
TGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTT
CTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAG
CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGC
ATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGCTGACG
TAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCG
GAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA
G

Scg9
UGUGAACAUACCACUUGUUGCCUCCGGCGSAUCRGCCCSCWCCACGGKRAAACGGGRCGGCCCGCCRGAGGA
MMCCUAAACTCTRTTTMYAYGAYGTMWCTTCTGAGTRRMACMAKCAAATAAWTCAAAACTTTYAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCRAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAATCTTTGAACGCACATTGCGCYCGCCAGYATTCTGGCGRGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCA
CMGCTTGGTKTTGGGRCYCSACGKYMRWUCGYGKKCCCTYAAAKKKAKTGGCGGWCCCTCCCGGAGCCTCCTTTG
CGTAGTAACTAACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACC
TCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAA

SCg10
GCGGAGGGATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGCAGG
GTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAAC
GACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCC
CGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACG
GCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTG
GGATCCGGAGGGACTCTTGCCGTAAAACCCCCAAUUUUCCAAAGGUUGACCUCGGAUCAGGUAGGAAUACCCG
CUGAACUUAAGCAUAUCAA

Scf11
GGGATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGCAGGGTCTCC
GTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGT
TTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGKTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAG
CATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGCTGAC
GTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCC
GGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTA
AGCATATCAATAA

Scf12
UGAGUUACCGCUCUAUAACCCUUUGUGAACGUACCUAACCGUUGCUUCGGCGGGCAGGGGAAGCCTCTCGC
GGGCCTCCCCTCCCGGCGCCGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACAC
GACGTCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCT
CGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACG
GCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTG
GGATCCGGAGGGACTCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGAATACCCGCT
GAACTTAAGCATAT

Scf13
ACTGTTGCTTCGGCGGGCAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAG
GATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTC
TTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
ATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCT
GCTTGGTGTTGGGGCCCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGT
AGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCG
GATCAGGTAGGAATACCCGCTGAACTTAAGCATAT

Scg14
ACATACCTATAACTGTTGCTTCGGCGGGCAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGC
GCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTA
ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA
GTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCA
ACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGA
GCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAA
AGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA

Scg15

AUUACUGAGUUUACGCUCUACAACCCUUUGUGAACAUACCUAUAACUGTTGCTTCGGCGGGCAGGGTCTCCG
TGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTT
CTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAG
CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGC
ATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGCTGACG
TAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCG
GAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA
G

SCg16

AACTGTTGCTTCGGCGGGCAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGA
GGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCT
CTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
AATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTC
TGCTTGGTGTTGGGGCCCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCG
TAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTC
GGATCAGGTAGGAATACCCGCTGAAACTTAAGCATATCAA

C.9 BLASTN result of representative isolates (Scg1-Scg10) recovered from infected material compared with the original isolates (C.g3, C.g2, and C.g1) obtained from naturally infected fruits

Query code	Query ID No.	Query length (bp)	Query identity	Subject code	Subject ID No.	Subject identity	% Query cover	% identity	Length (bp)	GenBank Accession No
Scg1	MT133276.1	588	Colletotrichum kahawae isolate BRD3-082M	C.g3	MT133276.1	*Colletotrichum kahawae* isolate BRD3-082M	100	100.00	588	MT133276.1
Scg2	MT083978.1	593	Colletotrichum gloeosporioides isolate ENF20	C.g3	MT133276.1	*Colletotrichum kahawae* isolate BRD3-082M	99	100.00	588	MT133276.1
Scg3	MN944922.1	591	Colletotrichum sp. strain LB8	C.g3	MT133276.1	*Colletotrichum kahawae* isolate BRD3-082M	95	90.53	588	MT133276.1
Scg4	MK646015.1	586	Colletotrichum fioriniae isolate F9	C.g3	MF495411.1	*Colletotrichum kahawae* isolate BRD3-082M	97	90.12	588	MT133276.1
Scg5	FJ539189.1	577	Colletotrichum gloeosporioides strain 268 18S	C.g3	MF495411.1	*Colletotrichum kahawae* isolate BRD3-082M	98	99.63	588	MT133276.1
Scg6	MF495411.1	599	Colletotrichum gloeosporioides strain LCM 871.01	C.g3	MF495411.1	*Colletotrichum kahawae* isolate BRD3-082M	97	100.00	588	MT133276.1
Scg7	KT282714.1	593	Colletotrichum sp.	C.g3	MF495411.1	*Colletotrichum kahawae*	99	100.00	588	MT133276.1

			ETHCTR09818S			isolate BRD3-082M				
Scg8	MT466533.1	553	Colletotrichum kahawae isolate BRD3-082M	C.g3	MT083978.1	*Colletotrichum kahawae* isolate BRD3-082M	99	90.50	588	MT133276.1
Sca9	MN871666.1	594	Colletotrichum fructicola strain KACC48896	C.g3	MT083978.1	*Colletotrichum kahawae* isolate BRD3-082M	96	90.36	588	MT133276.1
Sca10	MN273080.1	556	Colletotrichum kahawae strain BL22	C.g3	MT083978.1	*Colletotrichum kahawae* isolate BRD3-082M	97	99.81	588	MT133276.1

C.10 BLASTN result of representative isolates (Sca1-Sca13) recovered from infected material compared with the original isolates (C.a4 and C.a5) obtained from naturally infected fruits

Query Code	Query ID	Subject Code	Subject ID	Identity	% query cover	% identity	Genbank accession No	Length (bp)
Sca1	MT466533.1	C.a4	AB042300.1	*Glomerella acutata* genes	100.00	100.00	AB042300.1	584
Sca2	MN856423.1	C.a4	AB042300.1	*Glomerella acutata* genes	97%	99.31%	AB042300.1	584
Sca4	MN856415.1	C.a4	AB042300.1	*Glomerella acutata* genes	99%	99.15	AB042300.1	584
Sca5	MN856423.1	C.a4	AB042300.1	*Colletotrichum acutatum* strain WZ-306	81%	100.00	AB042300.1	591
Sca6	MG554643.1	C.a4	AB042300.1	*Glomerella acutata* genes	97%	99.48%	AB042300.1	584
Sca7	MN856423.1	C.a4	MT133290.1	*Colletotrichum acutatum* strain D215	81%	100.00 %	AB042300.1	589
Sca9	MG553373.1	C.a4	MT133290.1	*Glomerella acutata* genes	99%	99.49	AB042300.1	584
Sca10	MH930414.1	C.a4	MN586423.1	*Glomerella acutata* genes	96%	99.66	AB042300.1	584
Sca11	MN944922.1	C.a4	MT133290.1	*Glomerella acutata* genes	98	99.66	AB042300.1	584
Sca13	LC435467.1	C.a4	MN586423.1	*Glomerella acutata* genes	95	99.96	AB042300.1	584