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# Cross infection of *Colletotrichum* species; a case study with tropical fruits

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Strains of *Colletotrichum* were isolated from the fruits of chili, coffee, longan, mango, papaya and rose apple, collected from orchards and markets in Laos and Thailand. Isolates were identified using morphological characters, colony growth rate, and confirmed with DNA sequence data analysis of combined multi-gene loci. Pathogenicity testing of ten strains representing five species of *Colletotrichum* was carried out on *Capsicum* sp. (chili), *Carica papaya* (papaya), *Citrus reticulata* (orange), *Eugenia javanica* (rose apple), *Mangifera indica* (mango) and *Psidium guajava* (guava) using a wound drop technique. Pathogenicity and potential for cross infectivity of *Colletotrichum asianum*, *C. cordylinicola*, *C. fructicola*, *C. saimense* and *C. simmondsii* were tested on the hosts. The *Colletotrichum* strains belonging to different species tested were generally shown to infect a wide host range.

**Key words** – anthracnose – fruit infection – pathogenicity

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## Introduction

*Colletotrichum* is one of the most economically important pathogenic genera causing anthracnose of fruits, affecting a wide range of hosts in the tropics and subtropics (Cai et al. 2009, Cannon et al., 2012; Damm et al., 2012; Fujinaga et al., 2012; Hyde et al. 2009a, Phoulivong et al. 2010a, Noireung et al. 2012, Weir et al., 2012; Yang et al. 2012a, b). The above-ground plant parts of crops as well as fruit trees can be affected by *Colletotrichum* anthracnose and in the case of fruit infection, there is a reduction in yield quantity or quality (Phoulivong et al. 2010a). Hosts of *Colletotrichum* species in Thailand include fruits such as chili (*Capsicum* sp.), guava (*Psidium guajava*), jujube (*Zizyphus mauritiane*), mango (*Mangifera indica*), papaya (*Carica papaya*) and rose apple

(*Eugenia javanica*) (Freeman & Shabi 2000, Peres et al. 2002, Ratanacherdchai et al. 2010, Sreenivasaprasad and Talhinhas, 2005). *Colletotrichum* species are cosmopolitan with either multiple species occurring on a single host or a single species occurring on multiple hosts (Sander & Korsten, 2003). Fungus-host relationships are broad, imprecise and often overlapping (Freeman & Shabi 2000). *Colletotrichum* species can infect many hosts and may adapt to new environments (Sanders & Korsten 2003, Photita et al. 2004), leading to serious cross infection problems in plant production. The study of pathogenic variability of *Colletotrichum* species is therefore important and the understanding of the host range of a particular pathogen may help in efficient disease control and management (Whitelaw-Weckert et al. 2007).

Artificial inoculation methods *in vitro* are commonly used to test the pathogenicity of a fungal species, as it is easy to control environmental conditions. Common inoculation methods for pathogenicity testing include drop inoculation, wound/drop inoculation (Kanchana-udomkan et al. 2004), micro injection, and spraying with high pressure guns (Cai et al. 2009, Lin et al. 2002, Sharma et al. 2005, Than et al. 2008a). The drop method involves transferring a spore suspension on to the surface of fruit and the wound/drop method involves wounding the surface of the fruit by pricking with a pin then placing a drop of fungal spore suspension on the wounded tissue. The wound/drop method is more favourable since wounding allows the pathogenic isolate internal access to the fruit and enhances infection. The wound/drop method has been shown to be useful to select resistant varieties of chili (*Capsicum annuum*) from susceptible varieties (Lin et al. 2002, Than et al. 2008a). Different hosts and stages of maturity are important to test the expression of resistance to *Colletotrichum* species. The interaction between fruit maturity stage and infection of colonisation may depend on the species of *Colletotrichum* (AVRDC 2002). Pathogenicity testing can provide data on the resistance of fungi to crops in plant breeding programs and is important to integrated disease management programs because using the resistant varieties can reduce the negative effects of chemical use on the environment (AVRDC 2002, Freeman et al. 1998, Wharton et al. 2004).

*Colletotrichum gloeosporioides sensu lato* has been listed to cause disease of a very wide range of hosts (Table 1) (Cannon et al., 2012; Damm et al., 2012; Fujinaga et al., 2012; Ratanacherdchai et al. 2007, Than et al. 2008a,c; Weir et al., 2012). This species (*sensu stricto*) has recently been epitypified with a living strain that has been sequenced with data deposited in GenBank (Cai et al. 2009). This has enabled researchers to compare their isolates of *Colletotrichum* with the *C. gloeosporioides* epitype. This has resulted in the description of several new species in the *C. gloeosporioides* species complex (Cai et al. 2009, Cannon et al., 2012; Damm et al., 2012; Fujinaga et al., 2012; Noireung et al. 2012,

Phoulivong et al. 2010a, Prihastuti et al. 2009, Weir et al., 2012, Wikee et al. 2011, Yang et al. 2009, Yang et al. 2012a). With the introduction of these new species it is important to establish whether they are host-specific or have a wide host range as this will have important implications in disease control and management. The objective of this study is to understand the host range and cross infection of *Colletotrichum* species that were isolated from fruit lesions in Laos and Thailand.

## Material and Methods

### Isolation of *Colletotrichum* species

*Colletotrichum* strains were isolated from anthracnose of infected fruits from orchards and local markets in Laos and Thailand. Isolation was carried out by two methods depending on fungal sporulation on the sample. Conidia were picked directly from sporulating samples and then cultured on water agar (WA). The *Colletotrichum* isolates were then transferred to plates of potato dextrose agar (PDA) (Abang 2003). Alternatively, isolates were obtained from fruit without visible sporulation by culturing three 5×5 mm<sup>2</sup> pieces of tissue taken from the margin of infected tissue on WA. Before culturing on WA, the surface of infected tissues was sterilized by dipping in 1% sodium hypochlorite for 3 minutes, and rinsing three times with sterile water. The growing edge of any fungal hyphae developing from the disease tissue was then transferred aseptically to PDA. Single spore isolation was carried out from sporulating lesions. Spore masses were picked up with a sterilized wire loop and streaked onto the surface of water agar followed by inoculation overnight. A germinated single spore was picked up with a sterilized needle and transferred onto PDA to obtain a pure culture following the procedure described by (Cai et al. 2009).

### Pathogenicity testing

Preparation of inoculum – *Colletotrichum* isolates from a range of hosts used for pathogenicity testing and their cross infection potential are listed in Table 2. Pure cultures of each isolate were grown on PDA for 14 days at 27-28°C under fluorescent light (12 hour light/dark cycle), to induce

**Table 1** *Colletotrichum* species causing anthracnose in Laos and Thailand and reported host range

Species	Hosts	Causing anthracnose	References
<i>C. acutatum</i>	<i>Capcicum annuum</i>	Fruit	Damm et al. (2012)
	<i>Carica papaya</i>	Fruit	Damm et al. (2012)
	<i>Coffea arabica</i>	Fruit	Damm et al. (2012)
	<i>Fragaria ananassa</i>	Fruit	Damm et al. (2012)
<i>C. asianum</i>	<i>Capcicum annuum</i>	Fruit	This paper
	<i>Eugenia javanica</i>		
<i>C. brevispora</i>	<i>Neoregalia</i> sp.	Leaf	Noireung et al. (2012)
	<i>Pandanus pygmaeus</i>	Leaf	
<i>C. brisbanense</i>	<i>Capsicum annuum</i>	Fruit	Damm et al. (2012)
<i>C. coccodes</i>	<i>Solanum tuberosum</i>	Fruit	Lees & Hilton (2003)
<i>C. cordylinicola</i>	<i>Capcicum annuum</i>	Fruit	Phoulivong et al. (2010b), This paper
	<i>Carica papaya</i>	Fruit	
	<i>Cordyline fruticosa</i>	Leaf	
	<i>Eugenia javanica</i>	Fruit	
	<i>Mangifera indica</i>	Fruit	
	<i>Syzygium jambos</i>	Fruit	
<i>C. cuscutae</i>	<i>Malus sylvestris</i>	Fruit	Damm et al. (2012)
<i>C. dematium</i>	<i>Eryngium campestre</i>	Leaf	Noireung et al. (2012)
	Apiaceae		
<i>C. floriniae</i>	<i>Vaccinium</i> sp.	Fruit	Damm et al. (2012)
<i>C. fruticicola</i>	<i>Capcicum annuum</i>	Fruit	Prihastuti et al. (2009), This paper
	<i>Carica papaya</i>	Fruit	
	<i>Coffea arabica</i>	Fruit	
	<i>Eugenia javanica</i>	Fruit	
	<i>Mangifera indica</i>	Fruit	
<i>C. gloeosporioides</i>	<i>Citrus sinensis</i>	Fruit	Cannon et al. (2012)
<i>C. godetiae</i>	<i>Citrus aurantium</i>	Fruit	Damm et al. (2012)
<i>C. horii</i>	<i>Diospyros kaki</i>	Fruit	Wikee et al. (2011)
<i>C. horii</i>	<i>Diospyros kaki</i>	Leaf	Phoulivong et al. (2010b)
<i>C. ignotum</i>	<i>Jasminum sambac</i>	Leaf	Wikee et al. (2011)
<i>C. jasminigenum</i>	<i>Jasminum sambac</i>	Leaf	Wikee et al. (2011)
<i>C. kahawae</i>	<i>Coffea arabica</i>	Leaf	Prihastuti et al. (2009)
<i>C. melonis</i>	<i>Cucumis melo</i>	Fruit	Damm et al. (2012)
<i>C. musae</i>	<i>Musa</i> sp.	Fruit	Weir et al. (2012)
<i>C. nymphaeae</i>	<i>Fragaria</i> sp.	Fruit	Damm et al. (2012)
<i>C. pyricocola</i>	<i>Pyrus communis</i>	Fruit	Damm et al. (2012)
<i>C. queenslandicum</i>	<i>Carica papaya</i>	Fruit	Weir et al. (2012)
<i>C. simmondsii</i>	<i>Capcicum annuum</i>	Fruit	Giblin et al.(2010), Weir et al. (2012), This paper
	<i>Carica papaya</i>	Fruit	
	<i>Citrus reticulata</i>	Fruit	
	<i>Cordyline fruticosa</i>	Leaf	
	<i>Eugenia javanica</i>	Fruit	
	<i>Mangifera indica</i>	Fruit	
<i>C. tamarilloi</i>	<i>Solanum betaceum</i>	Fruit	Damm et al. (2012)
<i>C. thailandicum</i>	<i>Hibiscus rosa-sinensis</i>	Leaf	Noireung et al. (2012)
	<i>Alocasia</i> sp.	Leaf	
<i>C. tropicicola</i>	<i>Citrus maxima</i>	Leaf	Noireung et al. (2012)
	<i>Paphiopedilum</i>	Leaf	
	<i>bellatolum</i>	Leaf	
<i>C. truncatum</i>	<i>Phaseolus lunatus</i>	Leaf	Yang et al. (2009)
	<i>Glycine max</i>		
	<i>Crotalaria juncea</i>		

sporulation (Than et al. 2008a,b, Cai et al. 2009). The spores were harvested by placing about 10 ml sterile water onto the culture and filtering the spore and mycelium suspension with two layers of cheese cloth. The spore density was adjusted to a concentration of  $1 \times 10^6$  spore/ml using a haemocytometer.

Preparation of hosts – Freshly harvested untreated, unwaxed, physiologically mature and unripe fruits were collected from the field or purchased from the market (Sanders & Korsten, 2003). The detached fruits were washed under running tap water for 60 seconds followed by surface sterilization by immersing the fruits in 70% ethanol for 3 minutes, 1% sodium hypochlorite solution for 5 minutes and then rinsing three times in sterile distilled water for 2 minutes and drying with sterile tissue paper and then air drying.

Inoculation – Surface sterilized fruits were placed in a plastic box with tissue paper then sprayed with sterilized water to maintain at least 95% relative humidity (Than et al. 2008a). The samples were inoculated using the wound/drop inoculation method (Lin et al. 2002) which included pin-pricking the fruits to a 1 mm depth with a sterile needle in the middle portion of fruit and then placing 6  $\mu$ l of conidia suspension onto the wound (Freeman & Shabi 1996, Than et al. 2008a,b). Control fruits were inoculated with 6  $\mu$ l of sterile distilled water. The inoculated samples were incubated in the containers at 28-30°C in a 12 hour light/dark cycle.

Fruits used in inoculation tests were chili (*Capsicum* spp.), guava (*Psidium guajava*), mango (*Mangifera indica*), papaya (*Carica papaya*) and rose apple (*Eugenia javanica*) with ten treatments (numbered A-J) and three replicates per fruit. Incubation duration was dependent on the nature of the fruit lesion development on fruits. Fruits were examined at five days for rose apple and papaya, seven days for chili, guava, orange and varying periods for other fruits. The infection was measured based on lesion development on the symptom on fruit.

Lesion development on fruit were assessed by measuring the disease area in centimeters on each fruit; data were analysed used analysis of variance ( $P < 0.05$ ) with

DMRT for multiple range tests from statistic software (Cai et al. 2009, Choi et al. 2011, 2006, Than et al. 2008a,b).

## Results

### Pathogenicity testing

All of the isolates were identified using morphological characters, colony growth rate, and confirmed with DNA sequence data. (Phoulivong et al. 2010a,b).

The development of anthracnose symptoms on different fruits was statistically compared based on percentage of lesion area from the fruit (Table 2). All strains of *Colletotrichum* infected the original host from which they were isolated.

The strain of *Colletotrichum asianum* isolated from coffee infected chili and rose apple, whereas the strain isolated from mango infected chili and mango. *Colletotrichum cordylinicola* strain from rose apples infected a wide host range whereas that isolated from *Cordyline fruticosa* infected only papaya. Strains of *C. fruticola* from coffee and papaya had the same host range, whereas the isolate from longan infected mango but not orange. The *C. siamense* isolate from coffee infected five hosts including orange and papaya although the isolate from chili did not infect the latter two fruits. The two isolates of *C. simmondsii* were both from papaya and both infected mango, chili, rose apple and papaya. However, one isolate also infected guava whereas the other infected orange but not guava.

### Discussion

The *Colletotrichum* species infected a wide host range, however, the strains behaved differently. For example, the strain of *C. cordylinicola* isolated from rose apple failed to infect leaves of *Cordyline fruticosa* (Phoulivong et al. 2010b) while the strains of *C. cordylinicola* isolated from *Cordyline fruticosa* failed to infect rose apple fruit. The strain from rose apple however infected various other fruits. This study is consistent with inoculation studies by (Sanders & Korsten 2003b) who showed that isolates of *C. gloeosporioides* from mango could produce



**Fig. 1** – Anthracnose symptoms on papaya after 5 days inoculation **A** *Colletotrichum asianum* isolated from coffee berries; **B** *C. asianum* from mango fruit; **C** *C. cordylinicola* from rose apple fruit; **D** *C. fructicola* from coffee berries; **E** *C. fructicola* from papaya fruit; **F** *C. fructicola* from longan fruit; **G** *C. siamense* from coffee berries; **H** *C. siamense* from chili fruit; **I** *C. simmondsii* from papaya fruit; **J** *C. simmondsii* from papaya fruit.

symptoms on other hosts such as guava, chili pepper and papaya. Although mango isolates of *C. gloeosporioides* were highly pathogenic when re-inoculated onto mango fruits, it is unclear why no symptom was produced on chili fruits by the mango isolates. This could possibly have been due to a lack of pathogenicity factors that could recognize chili fruit cells for infection and colonization (Than et al. 2008a,b, Sanders & Korsten 2003b). The latter finding is extremely interesting as it shows that the same species isolated from different hosts, has different cross infection ability and this should be considered when establishing new species. There have been several studies concerning cross infection of *Colletotrichum* species especially with *C. acutatum* and *C. gloeosporioides* species complexes (Abang, 2003, Freeman et al. 2001, Kim et al. 2009, Peres et al. 2008, Sanders and Korsten 2003). Cross-infection of different hosts has not only been shown in the laboratory, but may also occur in the field (Afanador-Kafuri et al. 2003). Freeman et al.

(2001) found that *C. acutatum* from strawberry was able to cause lesions on various fruits. *In vitro* infection studies by (Whitelaw-Weckert et al. 2007) revealed low host-specificity among isolates of *C. acutatum*. Cross inoculation studies by Sanders & Korsten 2003, showed that putative isolates of *C. gloeosporioides* from mango could produce symptoms on other hosts such as guava, chili and papaya. These studies showed that *Colletotrichum* strains can infect more than one host and one host also can be infected with many *Colletotrichum* species.

Identification of strains in cross infection studies prior to 2010, and even many since were based on names given using data available at the time. It has now been shown that *C. acutatum* (Cannon et al., 2012; Talhinas et al. 2010, Damm et al., 2012; Fujinaga et al., 2012) *C. boninense* (Chong et al. 2011, Tarnowski & Ploetz, 2010, Weir et al., 2012) *C. gloeosporioides* (Cannon et al., 2012; Damm et al., 2012; Fujinaga et al., 2012; Weir et al., 2012, Živkovic et al. 2010) and

**Table 2** Pathogenicity testing and potential of cross infection of *Colletotrichum* species on a range of hosts

Species	Isolate Number	Species type	Hosts	Location	Infection on inoculated fruits					
					Orange	Guava	Mango	Chili	Rose apple	Papaya
					Infected fruit area (cm <sup>2</sup> )					
<i>C. asianum</i>	MFU090229	Holotype	coffee	Chiang Mai, Thailand	-	-	-	0.4BC*	1.33AB	-
<i>C. asianum</i>	MFU09 0556	-	mango	Bangkok, Thailand	-	-	1.25AB	0.2C	-	-
<i>C. cordylinicola</i>	MFU090551	Holotype	Cordyline	Chiang Mai, Thailand	-	-	-	-	-	0.35
<i>C. cordylinicola</i>	MFU090638	-	rose apple	Vientiane, Laos	-	0.5A	0.7AB	1A	1.73A	1.95A
<i>C. fructicola</i>	MFU090227	Holotype	coffee	Chiang Mai, Thailand	1.75A	-	-	0.75AB	1.40AB	1.5B
<i>C. fructicola</i>	MFU09 0560	-	papaya	Chiang Mai, Thailand	2A	-	-	0.75AB	1.07BC	1.45B
<i>C. fructicola</i>	MFU09 0568	-	longan	Chiang Mai, Thailand	-	-	1.95A	0.75AB	0.93BC	1C
<i>C. siamense</i>	MFU090230	Holotype	coffee	Chiang Mai, Thailand	1B	0.65A	0.3B	0.5BC	-	1C
<i>C. siamense</i>	MFU09 0548	-	chili	Luang Pra Bang, Laos	-	0.4A	0.4B	1A	-	-
<i>C. simmondsii</i>	BRIP28519	Holotype	papaya	Australia	-	0.35A	1.7AB	0.5BC	0.83C	1C
<i>C. simmondsii</i>	CBS.294.67	Epitype	papaya	Australia	1.5A	-	1.1AB	0.5BC	1.00BC	1C
LSD (between group)					0.49	0.31	1.41	0.44	0.44	8.1

\*Means with the same letter in each column are not significantly different from each other based on DMRT test in Sirichai statistics version 6; -, no infection.





**Fig. 2** – *Colletotrichum* symptoms on rose apple 5 days after inoculation **A** *C. asianum* isolated from coffee berries; **B** *C. asianum* from mango fruit; **C** *C. cordylinicola* from rose apple fruit; **D** *C. fructicola* from coffee berries; **E** *C. fructicola* from papaya fruit; **F** *C. fructicola* from longan fruit; **G** *C. siamense* from coffee berries; **H** *C. siamense* from chili fruit; **I** *C. simmondsii* from papaya fruit; **J** *C. simmondsii* from papaya fruit.

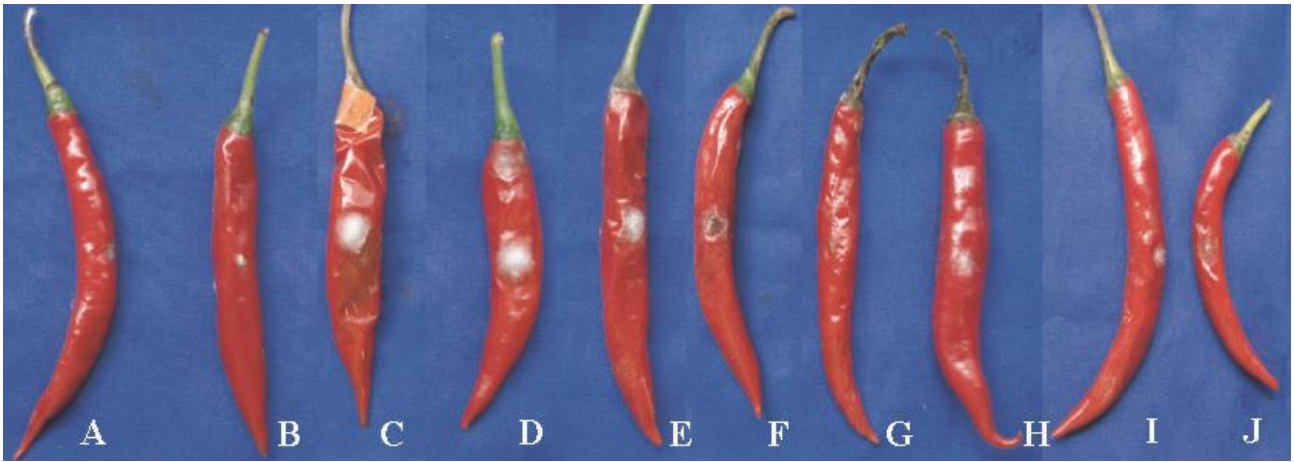
several other taxa are species complexes (Damm et al. 2012, Stankova et al. 2011, Weir et al. 2012). We therefore cannot compare our results with previous studies, as it is unlikely we were studying the same species.

Some recent studies have used strains that have been accurately identified based on combined sequence data. Phoulivong et al. (2010) showed that *C. asianum*, *C. fructicola*, *C. siamense* and *C. simmondsii* can infect chili, guava, jujube, mango, papaya and rose apple; Yang et al. (2012a) showed that *C. orchidearum*, *C. karstii* and *C. siamense* are not host-specific as they infected fruit of apple, chili and tomato following pathogenicity testing. Peng et al. (2012) showed that *C. boninense*, *C. brevisporum*, *C. fructicola*, *C. gloeosporioides*, *C. karstii*, *C. simmondsii* and *C. murrayae* infected citrus leaves, while Noireung et al. (2012) found that *C. brevisporum*, *C. tropicicola* and *C. thailandicum* caused anthracnose on leaves of *Pandanus pygmaeus*, *Citrus maxima* and *Hibiscus rosa-sinensis*. Most studies, including the present one, confirm that most *Colletotrichum* species have wide host ranges (Cai et al. 2010, Noireung et al. 2012, Phoulivong et al. 2010b, Yang et al. 2012b). Infection of fruits may be dependent on environmental factors such as variety and condition of the fruit, humidity and

temperature, and the concentration of inoculum (Simmonds 1965, Freeman et al. 1998), rather than which *Colletotrichum* species colonizes it.

Because pathogenicity testing involves wounding fruits, the results of this study may not accurately reflect the virulence potential of the strains (Phoulivong et al. 2010b, Weir et al. 2012). This study provides further evidence that most *Colletotrichum* species are not host-specific. However, some species of *Colletotrichum* have narrow host ranges. For example *C. kahawae* infects only coffee, *C. coccodes* infects on tomato and potato, *C. falcatum* infects only sugarcane, and *C. musae* infects only banana (Canon et al. 2008, Freeman et al. 2001, Kim et al. 2009, Prihastuti et al. 2009, Sreenivasapradad & Talhinhas, 2005, Yang et al. 2012b). Only some isolates of *C. kahawae* are able to cause coffee berry disease, and are therefore of biosecurity importance (Silva et al. 2012a,b) and these isolates could be distinguished using GS sequences (Weir et al. 2012), Apn25L and MAT 1-2-1 (Silva et al. 2012b).

From a quarantine perspective, it is important to establish the host range of a specific *Colletotrichum* species, as spread of host-specific taxa such as *C. kahawae* should be restricted. *Colletotrichum simmondsii*, *C. fructicola* and *C. siamense* can infect many fruits including chili, coffee, dragon fruit,

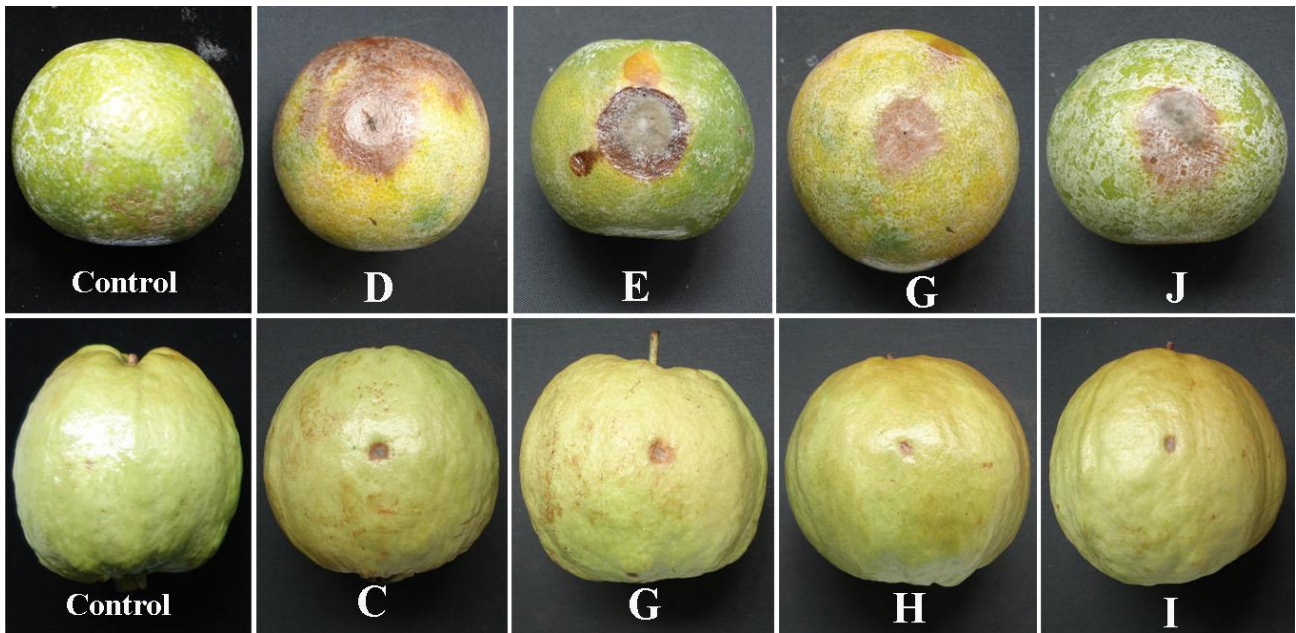


**Fig. 3** – Anthracnose symptom on chili 7 days after inoculation **A** *Colletotrichum asianum* isolated from coffee berries; **B** *C. asianum* from mango fruit; **C** *C. cordylinicola* from rose apple fruit; **D** *C. fructicola* from coffee berries; **E** *C. fructicola* from papaya fruit; **F** *C. fructicola* from longan fruit; **G** *C. siamense* from coffee berries; **H** *C. siamense* from chili fruit; **I** *C. simmondsii* from papaya fruit; **J** *C. simmondsii* from papaya fruit.



**Fig. 4** – Anthracnose symptom on mango 7 days after inoculation: **A** control; **B** *C. asianum* from mango fruit; **C** *C. cordylinicola* from rose apple fruit; **F** *C. fructicola* from longan fruit; **G** *C. siamense* from coffee berries; **H** *C. siamense* from chili fruit; **I** *C. simmondsii* from papaya fruit; **J** *C. simmondsii* from papaya fruit.





**Fig. 5** – Anthracnose symptoms on selected orange (i.e. D, E, G, J and control) and guava (i.e. C, G, H, I and control) 7 days after inoculation: **D** *C. fructicola* from coffee berries; **E** *C. fructicola* from papaya fruit; **G** *C. siamense* from coffee berries; **J** *C. simmondsii* from papaya fruit; **C** *C. cordylinicola* from rose apple fruit; **G** *C. siamense* from coffee berries; **H** *C. siamense* from chili fruit; **I** *C. simmondsii* from papaya fruit.

guava, mango, papaya, rose apple and strawberry (Phoulivong et al. 2010a, Table 2).

In Table 1 we list the species used in this study and their potential to infect various hosts, where species were identified based on molecular data. Strains of *Colletotrichum asianum* infected chili, mango and rose apple host and strains of *C. fructicola* infected chili, citrus, rose apple, and papaya. *Colletotrichum cordylinicola* was specific to *Cordyline fruticosa* leaves. It is therefore apparent that *C. asianum*, *C. fructicola*, *C. siamense* and *C. simmondsii* have wide host ranges, while *C. cordylinicola* has a narrow host range. This is important for understanding the ability of *Colletotrichum* species to infect different hosts (Stankova et al. 2011).

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