Received: 16 July 2023 | Accepted: 14 March 2024

DOI: 10.1111/ppa.13901

Plant Pathology WILEY

ORIGINAL ARTICLE

The pathogenic diversity and host range of *Colletotrichum* spp. causing pepper spot and anthracnose of lychee (*Litchi chinensis*) in Australia

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Funding information
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Abstract

Lychee pepper spot, a field disease affecting lychee fruit skin, pedicels and petioles, is caused by *Colletotrichum siamense*, a fungal pathogen within the gloeosporioides species complex. Members of *Colletotrichum* from the gloeosporioides species complex and occasionally those from the acutatum species complex also cause postharvest anthracnose of lychee. Pepper spot was first described in Australia many years after anthracnose on lychee was first described, giving rise to the hypothesis that a novel species or strain within the gloeosporioides species complex causes pepper spot. In the present study, 19 isolates of *Colletotrichum* spp., collected from pepper spot and anthracnose symptoms on lychee fruit, representing 13 different genotypes across five species, were inoculated onto lychee fruit in the field or on detached fruit in the laboratory, to understand more about their pathogenic diversity. We found that symptoms were specific to genotype of the pathogen, as three genetically similar isolates of *C. siamense* consistently caused pepper spot and anthracnose, whilst other isolates caused anthracnose only. Cross-inoculation studies on detached fruit of lychee, banana, avocado and mango also provided some evidence of host specialization in isolates of *C. siamense* infecting lychee in Australia. Our experiments provided further evidence that detached fruit assays cannot be used as a reliable proxy for field inoculation studies. This research confirms that *C. siamense* is a causal agent of both lychee pepper spot and lychee anthracnose in Australia, and *Colletotrichum alienum* and *Colletotrichum queenslandicum* are reported as causal agents of anthracnose of lychee for the first time.

KEYWORDS
anthracnose, *Colletotrichum siamense*, gloeosporioides species complex, host specialization, pepper spot

1 | INTRODUCTION

The lychee or litchi (*Litchi chinensis* subsp. *chinensis*) tree produces a highly valued fresh fruit (Nakasone & Paull, 1998), and in Australia, the industry is valued at AU$32 million (Hort Innovation, 2022). Lychees are produced predominantly along the east coast from Cooktown in north Queensland (15.47° S, 145.25° E) to Coffs Harbour in northern New South Wales (30.30° S, 153.11° E) (Diczbalis, 2012). Fruit is produced both for domestic consumption and also to supply international markets, with the demand from international markets particularly high during the Lunar New Year (Menzel & McConchie, 1998).

One of the most common postharvest diseases of lychee is anthracnose (Coates et al., 2005). It is caused by species of *Colletotrichum* from the gloeosporioides species complex and occasionally the acutatum species complex (Coates et al., 2005). Infection takes place in the field but generally remains quiescent until after harvest when symptoms appear as a browning of the pericarp (skin) (Anderson & Coates, 2009). The symptoms are usually limited to the pericarp and the aril is not affected but occasionally the infection will progress into the flesh causing collapse, and its external appearance downgrades the fruit at market (Fitzell & Coates, 1995).

Pepper spot is another disease of lychee that is caused by a member of the gloeosporioides species complex, *Colletotrichum siamense* (Ling et al., 2019; Ni et al., 2017). The first report of lychee pepper spot in Australia was in 1982, and by 1989 it had been found in all the major growing areas (Drew & Drew, 2001). Symptoms appear as small (<1 mm diameter) raised black lesions on the surface of fruit, leaves and petioles (Cooke & Coates, 2002) as if the fruit had been sprinkled with black pepper. These symptoms are often more severe on the lower branches of the affected tree compared with the upper branches (Drew & Drew, 2001). Pepper spot does not affect flesh but downgrades fruit appearance (Cooke & Coates, 2002) and adds additional cost to sort second-grade fruit. In Australia, pepper spot affects a number of lychee cultivars, namely Kwai May Pink, Bengal, Salathiel, Wai Chee and Tai So (Drew & Drew, 2001). Unlike anthracnose, there is not an extended quiescent period during the infection process on fruit, with symptoms appearing prior to harvest (Anderson & Coates, 2009). Tree nutrition has been suggested to play a role in lychee pepper spot development. In an early study by Drew and Drew (2001), trees without pepper spot had on average 48% higher leaf calcium levels than trees with pepper spot symptoms.

Symptoms similar to lychee pepper spot have also been recorded on avocado and are caused by several *Colletotrichum* species. In Australia, avocado pepper spot has been recorded on cv. Hass (Willingham et al., 2000) and in South Africa on cv. Hass and cv. Pinkerton (Schoeman & Manicom, 2000). The symptoms on avocado are similar to those seen on lychee, where the lesions appear as small (0.1–0.5 mm diameter) black raised necrotic zones on the surface of fruit, twigs and pedicels (Pegg et al., 2002; Willingham et al., 2000). Avocado pepper spot symptoms are often associated with fruit sunburn (Schoeman & Manicom, 2000), generally on the northern, more sun-exposed side of trees (in the southern hemisphere) (Giblin, 2006; Pegg et al., 2002), and are associated with conditions of high rainfall, high minimum temperatures and high minimum humidity, which prolongs the wet period of the fruit following rain (Schoeman & Manicom, 2000).

The use of DNA amplification fingerprinting (Giblin, 2006) and multilocus sequencing (Giblin et al., 2018) were unable to differentiate isolates originally collected from either anthracnose or pepper spot lesions of avocado. However, pathogenicity testing conducted by Giblin et al. (2010) demonstrated some differences between pepper spot and anthracnose isolates. Isolates originally from both symptom types could cause pepper spot on immature Hass fruit (approx. 2 cm in length at time of inoculation) in the field; however, the severity was significantly higher on fruit inoculated with isolates originally from pepper spot lesions than from anthracnose lesions (Giblin et al., 2010). In detached avocado fruit assays, pepper spot and anthracnose isolates caused similar levels of anthracnose but assessment of postharvest anthracnose on field-inoculated fruit was not undertaken (Giblin et al., 2010).

A genetic diversity study of 141 isolates of *Colletotrichum* spp. from lychee in Australia, as with the avocado studies, was unable to differentiate pepper spot from anthracnose isolates (Anderson et al., 2013). There were 21 genotypes defined as having identical arbitrarily primed PCR (ap-PCR) fingerprints based on 85 polymorphic markers. The majority of the isolates were classed as one of only two genotypes, Genotype 1 (35 isolates) and Genotype 6 (60 isolates); each genotype contained isolates from both pepper spot and anthracnose symptoms.

Host specialization within the gloeosporioides species complex has been demonstrated in previous studies. Using detached fruit assays, Hayden et al. (1994) showed that isolates from mango were highly aggressive on mango fruit but less aggressive on other fruit hosts tested such as banana, pawpaw and strawberry, thus indicating a mango biotype of *Colletotrichum gloeosporioides*. Subsequently, multilocus sequence typing has identified the causal agent of mango anthracnose as *Colletotrichum asianum* (Giblin et al., 2018).

The primary aims of this study were to investigate the association between genotype, species and pathogenicity of isolates of *Colletotrichum* spp. from lychee, and to examine if isolates from pepper spot and anthracnose could be distinguished on the basis of symptoms arising from field inoculations and detached lychee fruit assays. Mango, banana and avocado fruits were inoculated with isolates of *Colletotrichum* spp. from lychee to investigate host specialization. The relationship between disease development on field-inoculated fruit and in detached fruit assays was also examined.

2 | MATERIALS AND METHODS

2.1 | Isolates and identification

Nineteen monoconidial isolates of *Colletotrichum* spp. that had been collected from lychee pepper spot and lychee anthracnose lesions by the authors and previously characterized into

genotypes (Anderson et al., 2013) were used to select representative isolates for pathogenicity testing in this study (Table 1). One isolate of *C. asianum* from mango, one isolate of *Colletotrichum musae* from banana and one isolate of *C. siamense* from avocado were obtained from the Brisbane (Queensland) Plant Pathology Herbarium (Brisbane Pathology, BRIP) for inclusion in the pathogenicity testing.

Given the current understanding of the gloeosporioides species complex, the characteristics used to previously identify the genotypes (morphology, ap-PCR fingerprint, and rDNA internal transcribed spacer region [ITS] and *β-tubulin* gene sequencing) (Anderson et al., 2013) are insufficient for identification to species rank. To address this, the *β-tubulin* (*BTUB*), *calmodulin* (*CAL*), *glutamine synthase* (*GS*), *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) genes and Apn2-Mat1-2 intergenic spacer and partial mating type (Mat1-2) (ApMAT) regions were sequenced (File S1; Table 2) and used to generate a phylogenetic species hypothesis based on genomic data as described below with reference sequences (De Silva et al., 2017; Sharma et al., 2013, 2017; Wang et al., 2021; Weir et al., 2012).

NCBI assembled datasets of *Colletotrichum* species (*C. aenigma*, *C. asianum*, *C. camelliae*, *C. fructicola*, *C. gloeosporioides*, *C. musae* and *C. siamense*) were downloaded to include in genomic analyses (Table S1). Genomes were annotated using FunAnnotate (Palmer & Stajich, 2019) with protein models from the annotated reference assembly of *C. gloeosporioides* (GCA_000446055), BUSCO models for Sordariomycetes and Augustus models for *Neurospora crassa*.

OrthoFinder v.1.0.6 (Emms & Kelly, 2019) with a DIAMOND search (Buchfink et al., 2015) was used to identify orthologous groups of genes. Single-copy orthologues identified in OrthoFinder were aligned with MAFFT (Katoh & Standley, 2013) and trimmed using Gblocks (Castresana, 2000). Nucleotides of the five barcoding genes from each assembly were extracted from genomes using a BLASTn search (command: -outfmt '6 sseq'), and these were aligned to reference sequences of *Colletotrichum* from GenBank and those sequenced in the present study. All aligned single-copy orthologues and barcoding genes were concatenated with FASconCAT-G (Kück & Longo, 2014).

The most likely tree was searched in IQTree v.2 (Minh, Schmidt, et al., 2020) with a model test for each partition (command -spp -m TEST), 10,000 ultrafast bootstraps (Minh et al., 2013), and genealogical concordance factors calculated from gene trees for each locus and applied to the concatenated topology (Minh, Hahn, & Lanfear, 2020). Relationships among orthologues were visualized with a neighbour net in SplitsTree v. 4.14.8, and recombination was tested by calculating the pairwise homoplasy index (PHI) for the entire dataset (Huson & Kloepper, 2005).

2.2 | Inoculum preparation

Isolates were grown on oatmeal agar for 6–11 days at approximately 24°C under a 12 h near-UV light (300–380 nm)/12 h darkness light regime. Inoculum suspensions of conidia were made by flooding the plates with sterile distilled water (SDW) and using a glass spreader to gently dislodge the conidia. The suspension was filtered through at least four layers of sterile gauze and the concentration adjusted using a haemocytometer. An inoculum concentration of 5×10^6 conidia/mL was used for all experiments. For field inoculation, conidial suspensions were prepared in 0.01% vol/vol Tween 80 (Sigma Aldrich).

2.3 | Inoculation of fruit in the field

The field inoculations were conducted in a netted commercial lychee orchard at Woombye in south-east Queensland (26.65° S, 152.97° E) with fruit of cv. Kwai May Pink. The orchard had no history of pepper spot, and different trees were inoculated each season. Panicles carrying at least six fruits were selected and tagged approximately 2 weeks prior to commercial harvestable maturity. Whilst still attached, the fruit were washed with tap water and then SDW to remove residual protectant fungicide (mancozeb) prior to inoculation. Each tagged panicle was inoculated by dipping into a conidial suspension for approximately 30 s and enclosed in a plastic bag containing a small wad of damp cotton wool to keep humidity high around the panicle. The plastic bags were covered with white paper bags to reflect excess heat. After 48 h, both the paper and plastic bags were carefully removed and fruit checked for symptom development after 2 weeks.

Isolates were selected for field inoculations in 2007 based on ap-PCR grouping (Anderson et al., 2013) to include a range of genotypes (Table 1). These genotypes included representatives from *C. alienum*, *C. fructicola*, *C. queenslandicum*, *C. siamense* and *C. simmondsii*. Inoculations were carried out on four trees where the eastern and western sides of each tree were treated as split plots giving a total of eight replicates. In 2008 and 2009, isolates selected for inoculations were modified (Table 1) according to results obtained from the 2007 trial; additionally, an isolate of *C. asianum* originally obtained from mango was included. Eight single replicate trees were inoculated, and panicles of fruit only on the eastern sides of the trees were inoculated.

Two weeks (2007) or 19 days (2008 and 2009) after inoculation, when commercially ripe, the fruit were harvested and transported to the laboratory in plastic crates covered with damp towels to prevent desiccation. Fruits were removed from the panicles and assessed for percentage of surface area affected by pepper spot. The fruits from each panicle were placed into a $9.5 \times 9.5 \times 4.0$ cm plastic punnet, wrapped with plastic film and incubated at 20°C in the dark. After 6 (2007) or 10 (2008 and 2009) days, the fruit were assessed visually for the development of anthracnose lesions as a percentage of surface area affected per fruit. At this stage, pepper spot lesions were obscured by the development of anthracnose and so were not assessed after incubation. Data were analysed using analysis of variance (ANOVA) (Genstat 9th Edition) and means separated using the LSD test at the 5% level of significance.

TABLE 1 Details of *Colletotrichum* species isolates used in the pathogenicity trials.

Isolate name[a]	BRIP[b]	Location[c]	Symptom[d]	Genotype[e]	Species	Experiments in which the isolate was included				
						Field inoculation tests		Detached fruit assays		
						2007	2008/2009	2007	2008	Host range tests
ALPS11	39368	NSW	Pepper spot	1[f]	*C. siamense*	Y	Y	Y	Y	Y
ALPS15	39372	NSW	Pepper spot	1[f]	*C. siamense*	–	Y	–	Y	–
ALAN11	39393	NSW	Anthracnose	1[f]	*C. siamense*	–	Y	–	Y	–
ALAN12	39394	NSW	Anthracnose	3[f]	*C. siamense*	–	Y	–	Y	–
RLPS24	48642	NQ 1	Pepper spot	6[f]	*C. siamense*	–	Y	–	Y	–
RLAN11	48756	NQ 1	Anthracnose	6[f]	*C. siamense*	–	Y	–	Y	–
RLPS33	48645	NQ 1	Pepper spot	9[f]	*C. siamense*	Y	Y	Y	Y	–
ALAN43	39410	NSW	Anthracnose	10	*C. alienum*	Y	–	Y	–	–
RLAN35	48687	NQ 1	Anthracnose	12	*C. queenslandicum*	Y	–	Y	–	–
GLPS23	48675	NQ 2	Pepper spot	13	*C. queenslandicum*	Y	Y	Y	Y	Y
GLPS12	48671	NQ 2	Pepper spot	14	*C. fructicola*	Y	Y	Y	Y	Y
GLAN14	48727	NQ 2	Anthracnose	14	*C. fructicola*	Y	–	Y	–	–
GLPS54	48771	NQ 2	Pepper spot	14	*C. fructicola*	Y	–	Y	–	–
ALAN33	39405	NSW	Anthracnose	16	*C. alienum*	Y	Y	Y	Y	–
RLPS45	48652	NQ 1	Pepper spot	18	*C. siamense*	Y	Y	Y	Y	–
GLAN15	48744	NQ 2	Anthracnose	21	*C. queenslandicum*	Y	Y	Y	Y	–
GLAN11	48724	NQ 2	Anthracnose	22	*C. simmondsii*	–	Y	–	Y	Y
GLAN24	48729	NQ 2	Anthracnose	23	*C. simmondsii*	Y	–	Y	–	–
GLAN51	48737	NQ 2	Anthracnose	23	*C. simmondsii*	Y	–	Y	–	–
BRIP 28734	28734	Gin Gin, Qld.	Mango anthracnose	n/a	*C. asianum*	–	Y	–	Y	Y
BRIP 25490	25490	Unknown, Qld.	Banana anthracnose	n/a	*C. musae*	–	–	–	–	Y
BRIP 45655	45655	Mt Tamborine, Qld.	Avocado pepper spot	n/a	*C. siamense*	–	–	–	–	Y

Note: BRIP28734 collected by R. D. Davis, BRIP25490 collected by M. Dale, BRIP45655 collected by F. R. Giblin, all other isolates collected J. M. Anderson.

[a]The first letter in isolate name indicates location collected, third and fourth letters indicate symptom isolated from; PS, pepper spot; AN, anthracnose.

[b]Brisbane (Queensland) Plant Pathology Herbarium (BRIP) number.

[c]Location collected from. NSW, Brooklet; NQ 1, Toobanna; NQ 2, Mena Creek; Qld., Queensland.

[d]Symptom on plant from which the isolate was collected.

[e]Genotype based on arbitrarily primed PCR (ap-PCR) and rDNA internal transcribed spacer (ITS) and *β-tubulin* sequencing (Anderson, 2011; Anderson et al., 2013).

[f]Indicates isolate is part of a single closely related clade with Genotypes 1–9.

TABLE 2 The name, GenBank accession numbers and species based on sequencing of these loci of isolates used in the pathogenicity testing.

	GenBank accession number					
Isolate name	*BTUB*	*CAL*	*GAPDH*	*GS*	ApMAT	Species
ALPS11	OR227677	OR209763	OR227662	OR209748	OQ606875	*C. siamense*
ALPS15	OR227681	OR209767	OR227666	OR209752	OQ606879	*C. siamense*
ALAN11	OR227673	OR209759	OR227658	OR209744	OQ606871	*C. siamense*
ALAN12	OR227670	OR209756	OR227655	OR209741	OQ606868	*C. siamense*
RLPS24	OR227669	OR209755	OR227654	OR209740	OQ606867	*C. siamense*
RLAN11	OR227674	OR209760	OR227659	OR209745	OQ606872	*C. siamense*
RLPS33	OR227668	OR209754	OR227653	OR209739	OQ606866	*C. siamense*
ALAN43	OR227682	OR209768	OR227667	OR209753	OQ606880	*C. alienum*
RLAN35	OR227676	OR209762	OR227661	OR209747	OQ606874	*C. queenslandicum*
GLPS23	OR227678	OR209764	OR227663	OR209749	OQ606876	*C. queenslandicum*
GLPS12	OR227680	OR209766	OR227665	OR209751	OQ606878	*C. fructicola*
GLAN14	OR227679	OR209765	OR227664	OR209750	OQ606877	*C. fructicola*
GLPS54	OR227672	OR209758	OR227657	OR209743	OQ606870	*C. fructicola*
ALAN33	OR227671	OR209757	OR227656	OR209742	OQ606869	*C. alienum*
RLPS45	OR227675	OR209761	OR227660	OR209746	OQ606873	*C. siamense*

Note: Isolates GLAN15, GLAN11, GLAN24 and GLAN51 were sequenced and identified as *C. queenslandicum* (GLAN15) or *C. simmondsii* (GLAN11, GLAN24 and GLAN51) as part of studies by Shivas and Tan (2009) and Shivas et al. (2016); hence, GenBank accession numbers were not generated as part of this study and are not listed in this table.

In 2007 and 2008, isolations onto streptomycin-amended potato dextrose agar (SPDA) were carried out on a subset of fruit to confirm Koch's postulates via reisolation of the pathogen with which fruit were inoculated.

2.4 | Detached fruit assays

In 2007, detached fruit assays were undertaken using the same representative isolates as those used in the field inoculation trial that year (Table 1). Ripe lychee fruit of cv. Kwai May Pink were harvested from multiple trees from a commercial orchard at Brooklet in northern New South Wales (28.72° S, 153.52° E) where fruit had only been treated with protectant fungicides. Fruits that were free of obvious blemishes and pepper spot, and distant from fruit with pepper spot, were harvested. Fruits were immediately packed into a cool ice box and transported to the laboratory.

Prior to inoculation, fruits were rinsed with tap water and then SDW and air dried for approximately 30 min before being arbitrarily assigned to plastic punnets for inoculation. Five fruits were placed in each punnet, and two punnets of fruit were assigned to each treatment (isolate). Punnets were placed in plastic boxes lined with moistened blotting paper and each fruit received a 25 μL droplet of a conidial suspension (5×10^6 conidia/mL as above) or SDW (as a control). The droplet was placed directly adjacent to a mark made by a permanent marker on each fruit.

The incubator boxes were sealed with tape to maintain high humidity and placed at 20°C in the dark. Forty-eight hours after inoculation, the punnets were removed from the incubator boxes, each wrapped with plastic film and replaced into the incubator. Four days later, when fruit started to develop disease symptoms, lesion diameters perpendicular to the stalk of the fruit were measured using Vernier callipers, and then daily for the next 8 days.

Area under disease progress curve (AUDPC) (Akinsanmi et al., 2007) was calculated for each isolate and compared among isolates using analysis of variance (Genstat 11th Edition). AUDPC data were compared with anthracnose data from the 2007 field inoculation trial using correlation analysis (Genstat 11th Edition).

In 2008, the detached fruit assays were repeated but with the isolates used in the 2008 field inoculation trial (Table 1). The procedures were as described for 2007 except fruits were collected from a commercial orchard in Woombye, and inoculated fruits were incubated for 60 h rather than 48 h. After lesions began to develop they were measured daily for 5 days.

2.5 | Host range test

To investigate host specialization of *Colletotrichum* spp., detached fruit assays were undertaken with several isolates on lychee, avocado, mango and banana fruit.

Ripe fruit of lychee cv. Salathiel were harvested from a farm at Yandina, south-east Queensland (26.55° S, 152.95° E) in February 2009. These fruits had only received protectant fungicide (mancozeb or copper) applications throughout the growing season, and natural field infection was reduced by dipping in hot water (50°C)

for 2 min after harvest based on a method of Scott et al. (1982), then cooled at room temperature for 15 min before being stored at 6°C for 18 h prior to inoculation.

Banana fruit of cv. Goldfinger (also known as cv. FHIA-01) were obtained from a plantation at Farrants Hill in northern New South Wales (28.32° S, 153.48° E) in February 2009. The fruit had not received fungicide applications but had been covered with plastic bunch covers soon after emergence to protect the developing fruit. Fruits were harvested when approximately 25% of the fruit skin was still green and kept at room temperature for 2 days prior to inoculation. Fruits were washed with warm tap water prior to inoculation to remove sap residues.

'Cocktail' avocados are fruit that have not formed a proper seed due to embryo abortion and grow to approximately 5–10 cm long. Cocktail fruit achieve full maturity and ripen after harvest (Newett et al., 2001). Cocktail avocados of greenskin cv. Fuerte that had not received any fungicide applications were harvested from a farm at Duranbah in northern New South Wales (28.30° S, 153.53° E) in May 2009. Fruits were inoculated on the day of harvest.

Mature green cv. Kensington Pride mangoes were harvested from a residential garden in Indooroopilly, Queensland (27.50° S, 152.98° E) in January 2010. The mangoes had not received fungicide treatments. Fruit were desapped by trimming the pedicel to 5 mm, inverting and allowing sap to drain for 30 min, then washed with warm tap water and mild detergent to remove sap residues.

The avocado, banana and mango fruits were placed directly in plastic boxes, and lychees were placed into punnets as described previously before being inoculated with 25 µL droplets of conidial suspensions of *Colletotrichum* spp. (Table 1) or SDW as control. For lychee inoculations, a replicate consisted of three fruits in a punnet, each fruit with a single droplet of inoculum. For banana, avocado and mango, three droplets of inoculum were applied within circles drawn on the fruit surface with a permanent marker pen. Each fruit constituted a single replicate. For lychee, banana and mango, there were four replicates per treatment. Due to concerns over high levels of natural infection, eight replicates were used for avocado.

For all fruit, boxes were sealed and incubated at 23°C for 72 h after which the fruit were removed from plastic boxes. Lychee punnets were wrapped with plastic film whilst bananas, avocados and mangoes were transferred to commercial cardboard boxes with plastic liners. All fruit were maintained at 23°C. The diameters of fruit lesions were measured 8 days after inoculation on lychee and when fruit were ripe for banana, avocado and mango. Data were analysed using ANOVA (Genstat 11th Edition) and means separated using the LSD test at the 5% level of significance.

3 | RESULTS

3.1 | Phylogenomic identification of isolates and population genomics

We annotated 62 genomes of *Colletotrichum* downloaded from GenBank (mean number of genes per genome 14,406) and identified 3144 single-copy orthologues. A combined dataset of single-copy orthologues and molecular barcodes of reference specimens of *Colletotrichum* recovered isolates from lychee as four different species, namely *C. alienum*, *C. fructicola*, *C. queenslandicum* and *C. siamense* (Figure 1a). A SplitsTree network of a reduced dataset was used to visualize the relationships among the gloeosporioides species complex (Figure 1b). Recombination among species of *Colletotrichum* is supported by high reticulation and a PHI of 0.0, which is evidence of randomness across the alignment. Strong evidence of clonality or near clones in species of *Colletotrichum* is supported by high genealogical concordance factors at nodes that contain genomes in the phylogenetic tree, and little genetic differentiation or reticulation within species based on branch length in the SplitsTree network.

3.2 | Field inoculation trial

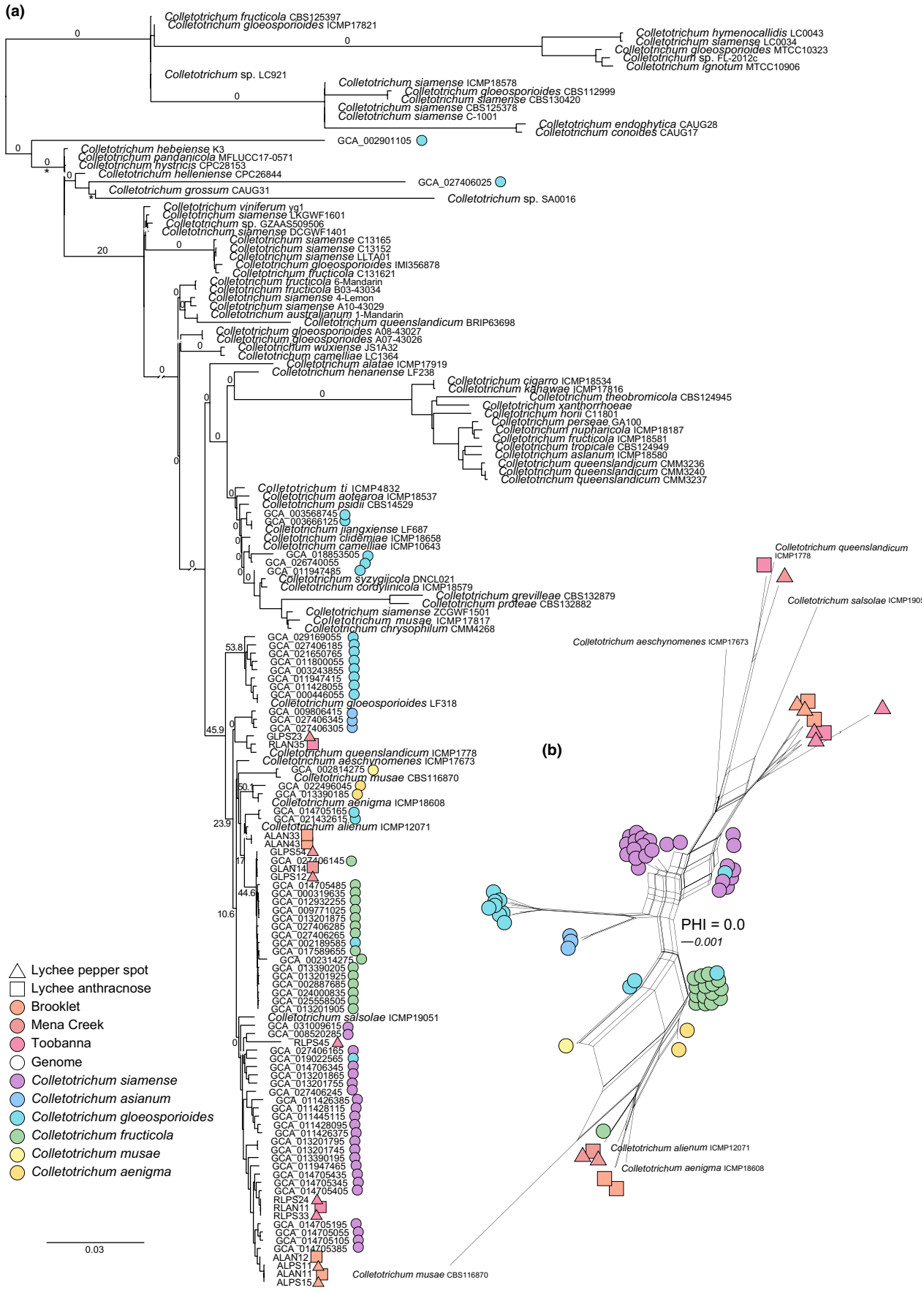
3.2.1 | 2007

No disease symptoms were observed when bags were removed from fruit 2 days after inoculation. At harvest (14 days post-inoculation), the only isolate to cause significantly more severe pepper spot compared to water-inoculated control and every other isolate was ALPS11 (*C. siamense* originally isolated from a pepper spot lesion) (Figures 2 and 3). ALPS11 and all other isolates caused significantly larger postharvest anthracnose lesions than the water control (Figure 3). The isolates included *C. siamense* (ALPS11, RLPS33, RLPS45), *C. queenslandicum* (RLAN35, GLPS23, GLAN15), *C. fructicola* (GLPS12, GLAN14, GLPS54), *C. alienum* (ALAN33, ALAN43) and *C. simmondsii* (GLAN24, GLAN51). Cultures with colony morphologies consistent with those of the inoculated isolates were obtained when symptomatic tissues were plated onto SPDA.

3.2.2 | 2008

Three *C. siamense* isolates (ALPS11, ALAN11 and RLPS24) caused levels of pepper spot significantly more severe than the

FIGURE 1 Relationships among species of *Colletotrichum* recovered from 3144 single-copy orthologue genes and five barcoding genes. (a) Phylogram recovered from a maximum-likelihood search with genealogical concordance factors as a percentage of the genes that recovered a node. Asterisks indicate branches that were not supported by 10,000 ultrafast bootstraps or 10,000 replicates of an approximate-likelihood ratio test. (b) SplitsTree NeighborNet visualizing relationships among the gloeosporioides species complex. Networks illustrate all possible relationships among data, with reticulation a signature of recombination and edge length informative of genetic distance. The pairwise homoplasy index (PHI) approaches 0.0 in recombinant populations and 1.0 in clonal populations.



water-inoculated control and all other isolates tested (Figure 4). ALAN11 and RLPS24 were not tested in the 2007 field trial. The five other isolates of *C. siamense* tested in 2008 (ALPS15, ALAN11, RLPS33, RLAN11 and RLPS45) did not cause any significant pepper spot symptoms. Pepper spot severity caused by the mango *C. asianum* isolate (BRIP 28734) and the other species, *C. queenslandicum* (GLPS23 and GLAN15), *C. alienum* (ALAN33), *C. fructicola* (GLPS12) and C. *simmondsii* (GLAN11) was not significantly different to the water controls (Figure 4).

The postharvest anthracnose disease from the 2008 field trial was more severe (Figure 4) compared with the 2007 field trial (Figure 2), most likely because fruit were incubated for 4 days longer in 2008. All isolates, except for mango *C. asianum* isolate BRIP 28734, caused more severe anthracnose than the water controls. These noninoculated fruits expressed low levels of disease due to natural infection not prevented by the protectant fungicide applications. The severity of anthracnose was not significantly different among *C. siamense* (ALPS11, ALPS15, ALAN11, RLPS24, RLAN 11, RLPS33, RLPS45), *C. queenslandicum* (GLPS23, GLAN15), *C. alienum* (ALAN33) and *C. simmondsii* (GLAN11). Cultures with colony morphologies consistent with those of the inoculated isolates were obtained when symptomatic tissues were plated onto SPDA.

FIGURE 2 Pepper spot on mature lychee fruit inoculated with isolate ALPS11 (*Colletotrichum siamense*) 2 weeks after inoculation.

3.2.3 | 2009

The 2009 field trial used the same isolates as the 2008 trial, and the results were very similar. The only isolates to cause significantly more pepper spot compared to water controls were ALPS11, ALAN11 and RLPS24 (all *C. siamense*) (Figure S1), which was consistent with the 2008 field trial results. These isolates along with ALPS15 (*C. siamense*), ALPS33 (*C. alienum*) and GLAN15 (*C. queenslandicum*) caused significantly greater levels of anthracnose than RLAN11, ALAN12, RLPS45 (*C. siamense*), GLPS23 (*C. queenslandicum*) and the mango *C. asianum* isolate BRIP 28734. Again, fruit inoculated with mango *C. asianum* isolate BRIP 28734 had less severe anthracnose than fruit inoculated with all other isolates and was not significantly different to the water-inoculated control. Interestingly, in 2009 GLPS12 (*C. fructicola*) caused more severe anthracnose than in 2008 and 2007.

3.3 | Detached fruit assays

The detached fruit inoculations resulted in anthracnose for the majority of the isolates. In the 2007 trial, lesions started to develop 2 days after inoculation. The isolate GLPS54 (*C. fructicola*) had the largest AUDPC for anthracnose (Figure 5) and was statistically greater than AUDPC for all of the other isolates except for

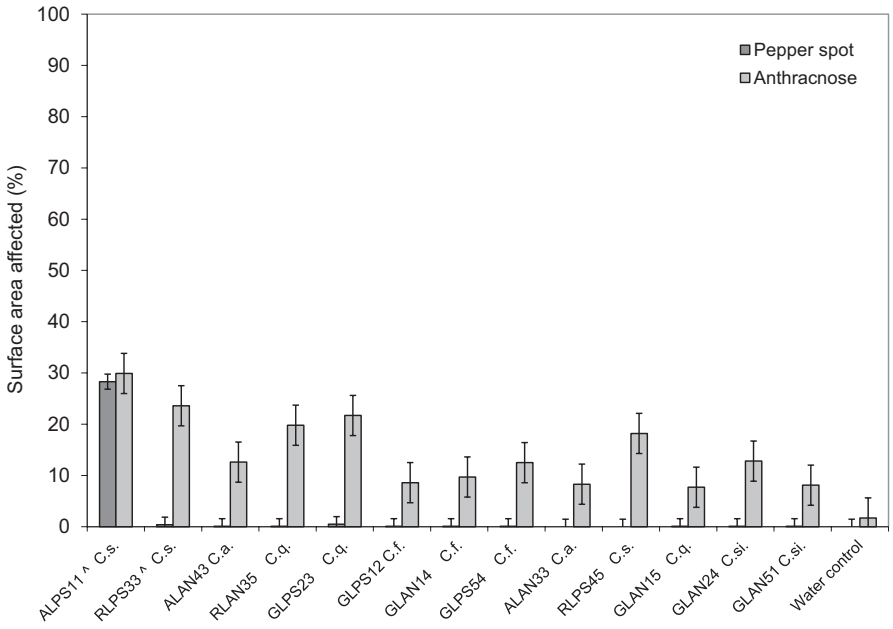


FIGURE 3 The mean percentage surface area affected by pepper spot (assessed at harvest) and anthracnose (assessed after 6 days' storage at 20°C) for fruit inoculated with isolates of *Colletotrichum* spp. 2 weeks prior to harvest in a field trial in 2007. Data were compared using analysis of variance ($n = 8$), and bars indicate least significant difference (LSD) at a 5% level of significance. Isolates marked with ^ are from a tight clade (Genotypes 1 to 9, Anderson et al., 2013). C.a., *C. alienum*; C.f., *C. fructicola*; C.q., *C. queenslandicum*; C.s., *C. siamense*; C.si., *C. simmondsii*.

RLAN35 (*C. queenslandicum*) and GLAN14 (*C. fructicola*). In most cases, there were no significant differences in AUDPC among isolates.

For all of the isolates tested in 2007, there was no correlation between the level of disease an isolate caused in field inoculations and in detached fruit assays (AUDPC) (correlation coefficient = 0.2187, $p = 0.453$). For example, the AUDPC (Figure 5) for GLPS54 (*C. fructicola*) was one of the largest whilst the postharvest anthracnose caused by the isolate in field inoculations was only moderate (Figure 2). RLPS45 (*C. siamense*) caused small lesions in the detached fruit assays but postharvest anthracnose of moderate severity from field inoculations.

In the 2008 detached fruit experiments, lesions started to develop 5 days after inoculation. The AUDPC for ALPS11 (*C. siamense*) was significantly greater than for any other isolate (Figure 6). ALAN33 (*C. alienum*) had the next largest AUDPC, and this was significantly greater than all of the isolates except ALAN11 (*C. siamense*). RLPS45, RLPS33, RLAN11 (*C. siamense*), GLPS12 (*C. fructicola*), GLPS23 (*C. queenslandicum*) and GLAN11 (*C. simmondsii*) all had AUDPCs that were not significantly different to each other. Fruit inoculated with RLPS24 (*C. siamense*) and water control fruit did not develop any lesions. The mango isolate of *C. asianum* BRIP 28734 caused moderate-sized lesions. In 2008, there was also no correlation between the field trial data and the detached fruit assays (correlation coefficient = 0.4472, $p = 0.095$).

3.4 | Host range test

On lychee fruit, inoculation with the isolate ALPS11 (*C. siamense*) caused the largest anthracnose lesions, which were significantly larger than those resulting from inoculations with all of the other

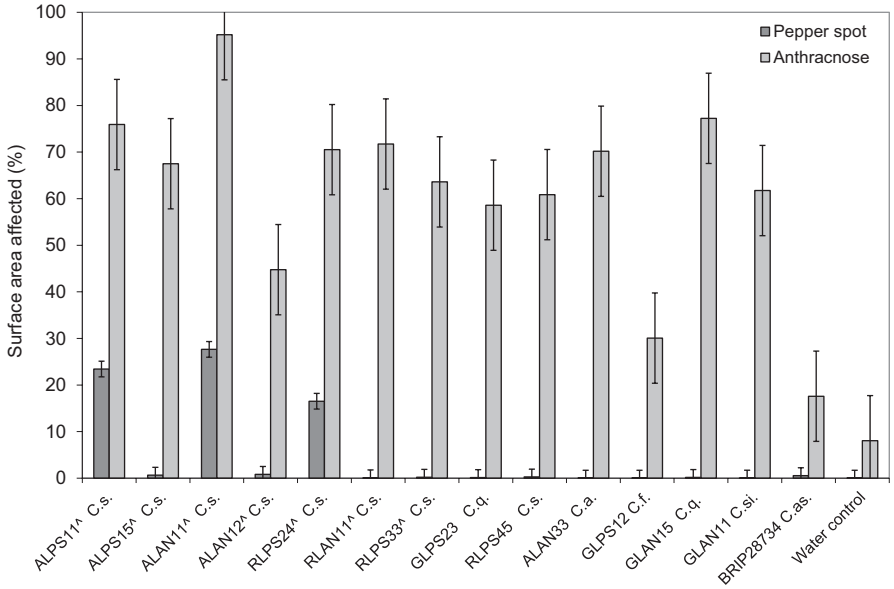


FIGURE 4 The mean percentage surface area affected by pepper spot (assessed at harvest) and anthracnose (assessed after 10 days' storage at 20°C) for fruit inoculated with isolates of *Colletotrichum* spp. 2 weeks prior to harvest in a field trial in 2008. Data were compared using analysis of variance ($n = 8$) and bars indicate LSD at a 5% level of significance. Isolates marked with ^ are from a tight clade (Genotypes 1 to 9, Anderson et al., 2013). C.a., *C. alienum*; C.as., *C. asianum*, C.f., *C. fructicola*; C.q., *C. queenslandicum*; C.s., *C. siamense*; C.si., *C. simmondsii*.

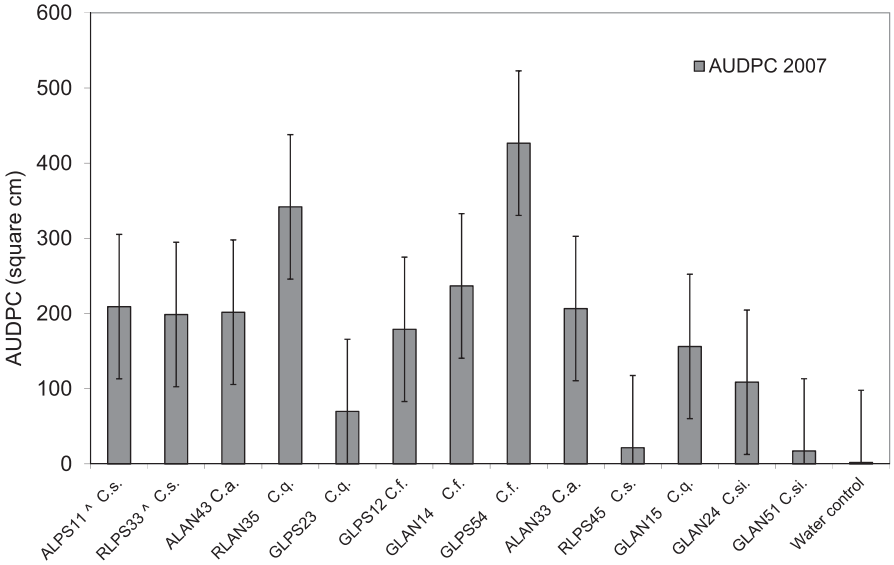


FIGURE 5 The area under disease progress curve (AUDPC) for anthracnose on detached fruit of lychee cultivar Kwai May Pink inoculated with *Colletotrichum* spp. isolates 11 days after inoculation. Data were compared using analysis of variance ($n = 10$), and bars indicate LSD at a 5% level of significance. Isolates marked with ^ are from a tight clade (Genotypes 1 to 9, Anderson et al., 2013). C.a., *C. alienum*; C.f., *C. fructicola*; C.q., *C. queenslandicum*; C.s., *C. siamense*; C.si., *C. simmondsii*.

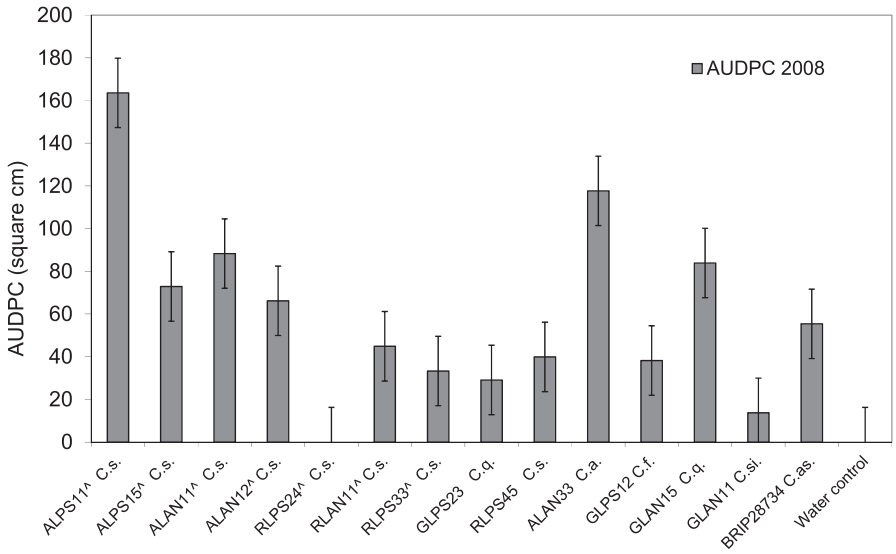


FIGURE 6 The area under disease progress curve (AUDPC) for anthracnose on detached fruit of lychee cultivar Kwai May Pink inoculated with *Colletotrichum* spp. isolates 10 days after inoculation. Data were compared using analysis of variance ($n = 10$), and bars indicate LSD at a 5% level of significance. Isolates marked with ^ are from a tight clade (Genotypes 1 to 9, Anderson et al., 2013). C.a., *C. alienum*; C.as., *C. asianum*; C.f., *C. fructicola*; C.q., *C. queenslandicum*; C.s., *C. siamense*; C.si., *C. simmondsii*.

isolates tested, except GLPS12 (*C. fructicola*) (Figure 7). The mango isolate of *C. asianum* (BRIP 28734) and avocado *C. siamense* (BRIP 45655) caused intermediate-sized lesions, and isolate GLAN11 (*C. simmondsii*) from lychee caused lesions slightly larger than, but similar to, GLPS23 (*C. queenslandicum* from lychee). The *C. musae* isolate BRIP 25490 did not cause high levels of disease, and mean lesion size was not significantly different to the control and GLPS23 (*C. queenslandicum*).

On avocado, the *C. siamense* BRIP 45655 (avocado) isolate caused the largest anthracnose lesions, which were not significantly different to those caused by GLPS12 (*C. fructicola*) and GLPS23 (*C. queenslandicum*). The size of the lesions on fruit inoculated with *C. musae* BRIP 25490, *C. asianum* BRIP 28734 (mango), GLAN11 (*C. simmondsii*) and ALPS11 (*C. siamense*) was not significantly different from the water control (Figure 7).

C. musae BRIP 25490 caused the largest lesions on banana, significantly larger than lesions on fruit inoculated with other isolates (Figure 7). The mango isolate of *C. asianum* BRIP 28734, GLAN11 (*C. simmondsii*), and ALPS11 (*C. siamense*) and GLPS12 (*C. fructicola*) from lychee caused lesions one-third the size of those caused by *C. musae*, which were not dissimilar to one another and were significantly larger than lesions on fruit from the water control or those inoculated with GLPS23 (*C. queenslandicum*) or *C. siamense* BRIP 45655 (avocado).

On mango, only the *C. asianum* (mango) isolate BRIP 28734 was able to cause lesions (Figures 7 and 8). The other isolates were not pathogenic on mango and, instead of anthracnose lesions, caused a hypersensitive-like response on the mango surface (Figure 8, inset).

4 | DISCUSSION

This paper is the first to verify that *C. siamense* is a causal agent of anthracnose and pepper spot on lychee fruit in Australia, and corroborates previous reports of pepper spot causal organisms from mainland China (Ling et al., 2019) and Taiwan (Ni et al., 2017).

C. alienum, *C. fructicola*, *C. queenslandicum* and *C. simmondsii* caused anthracnose on lychee fruit in field inoculations and were reisolated from inoculated fruit. This is the first time that *C. alienum* and *C. queenslandicum* have been demonstrated to cause anthracnose of lychee. Previously Shivas et al. (2016) had reported *C. queenslandicum* associated with lychee fruit and *C. tropicale*, a species genetically close to *C. siamense*, had been isolated from lychee leaves in Japan (Weir et al., 2012). *C. simmondsii* (recorded as *C. acutatum*) has previously been recorded as a causal agent of lychee anthracnose in Australia (Johnson et al., 2002). Li et al. (2021) reported *C. fructicola*, along with *C. siamense*, *C. asianum*, *C. musae*, *C. kahawae* subsp. *kahawae* and *C. horri*, as causes of lychee anthracnose in Hainan, China.

Species of *Colletotrichum* in the gloeosporioides species complex are probably near clones (Taylor et al., 2015; Tibayrenc & Ayala, 2012). There is little genetic diversity within species based on over 3000 protein-coding genes, and there is evidence of recombination among species based on reticulation and the PHI. Under the current taxonomic hypothesis for *Colletotrichum*, species are host-delimited populations that reproduce asexually more frequently than sexually. Future studies could examine mating compatibility or gene flow between different populations to better understand how recombination could change pathogenicity in *Colletotrichum*.

The relationships of the genotypes identified with ap-PCR (Anderson et al., 2013) were generally congruent with the topology of the species phylogenetic hypothesis from phylogenomic data and barcoding genes. For example, ALPS11, ALPS15, ALAN11 and ALAN12 (Genotypes 1 and 3), and RLPS24, RLAN11 and RLPS33 (Genotypes 6 and 9) were identified as two separate groups of *C. siamense*. The *C. fructicola* isolates (GLPS12, GLAN14 and GLPS54) also separated in the ap-PCR analysis into Genotype 14, a genotype in which 14 out of 15 monoconidial isolates formed the teleomorph in culture (Anderson et al., 2013). However, the results for the ap-PCR and phylogenetic analysis did not concur for all isolates; this is unsurprising given the different scale and

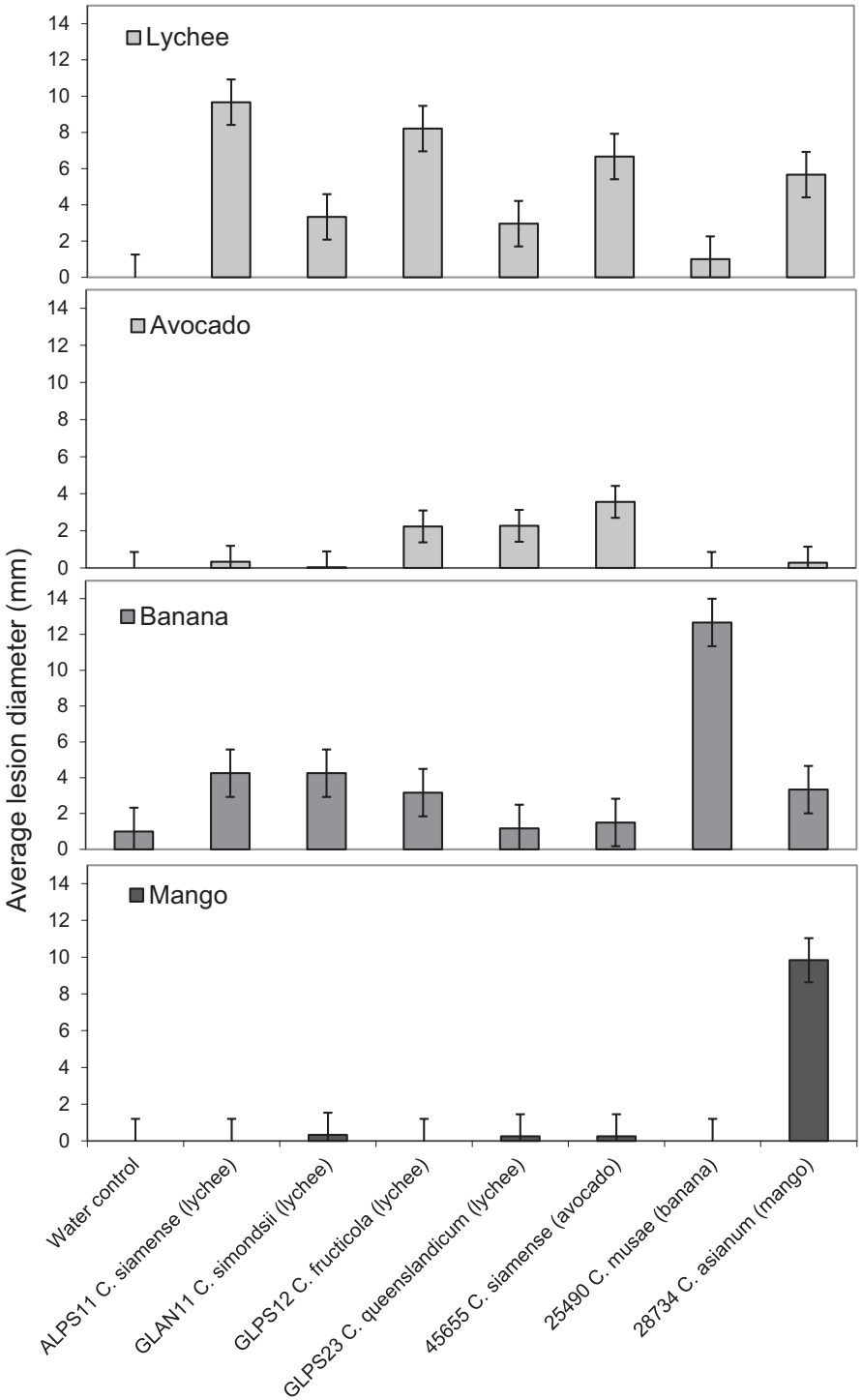


FIGURE 7 Mean lesion diameter on lychee, avocado, banana and mango fruit inoculated with seven isolates of *Colletotrichum* spp. Data were analysed using analysis of variance ($n = 4$ for lychee, banana and mango, $n = 8$ for avocado). Bars indicate LSD at a 5% level of confidence.

purposes of the two methods employed to investigate genetic diversity. The ap-PCR markers were used to select representative isolates from a collection of 150 isolates for pathogenicity testing whilst the phylogenetic analysis was used to assign the isolates to species.

The work presented in this paper demonstrates that pepper spot is caused by specific isolates of *C. siamense* and not due purely to a host response, such as stress. Across all of the field studies, three of the seven closely related isolates from one phylogenetic clade consistently caused pepper spot in field trials. These isolates consistently caused pepper spot across multiple years; for example, ALPS11 caused pepper spot on fruit in three field trials and ALAN11 and RLPS24 caused pepper spot in two field trials. This indicates that the ability to form pepper spot lesions is under genetic control and that there is evidence for a novel genotype of *C. siamense* causing pepper spot. Giblin et al. (2018) described *C. siamense* as the most commonly recovered species from avocado fruit with either pepper spot or anthracnose. Pathogenicity studies demonstrated that pepper spot and anthracnose isolates differed in the severity of pepper spot they caused in leaf petiole

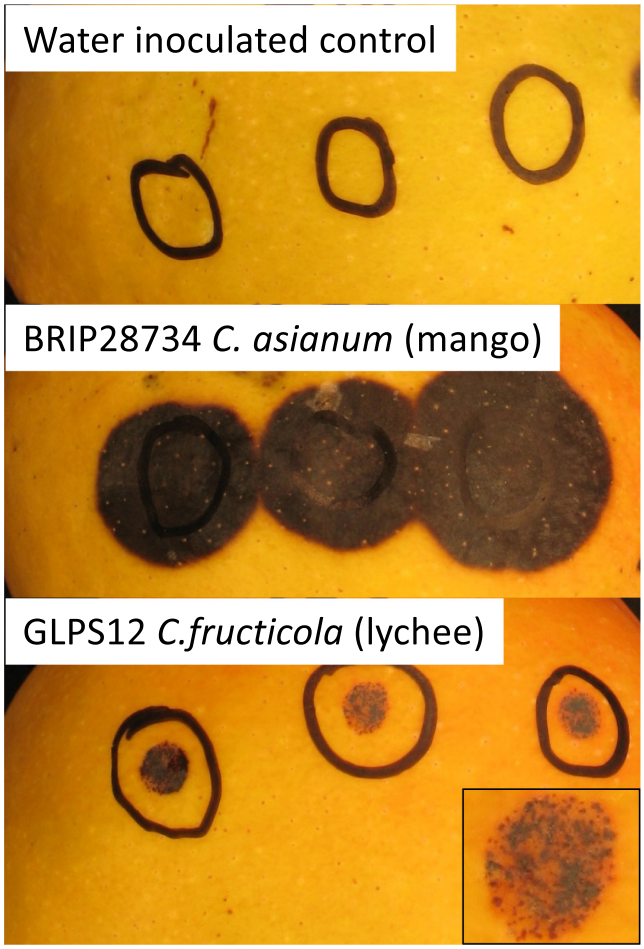


FIGURE 8 Mango cross-pathogenicity study 15 days after inoculation. The fruit inoculated with mango anthracnose isolate BRIP 28734 (*Colletotrichum asianum*) has large lesions, whilst there are hypersensitive-like lesions on the fruit inoculated with lychee isolate GLPS12 (*Colletotrichum fructicola*) (detail shown in inset).

assays in the glasshouse and on immature cv. Hass fruit in the field (Giblin et al., 2010).

Some isolates originally obtained from lychee pepper spot lesions, namely, RLPS45 (*C. siamense*), GLPS12 and GLPS54 (*C. fructicola*), did not cause pepper spot in field inoculation studies. Given the quiescent phase of *Colletotrichum* spp., it is not possible to exclude the chance of collecting an isolate that had not caused the disease symptom. When isolations were made from the pepper spot lesions, it is possible an 'anthracnose' isolate from a quiescent infection was isolated as well, and when single germinated conidial isolates were generated, the anthracnose isolate could have been selected. It is not unusual for more than one isolate or species to be present in close proximity on leaf and fruit surfaces. Fitzell (1981) found *C. gloeosporioides* and *C. acutatum* could be cohabitants of the same lesion on a mango leaf.

Whilst some isolates obtained from pepper spot lesions were unable to cause pepper spot in pathogenicity testing, ALAN11 (*C. siamense*), an isolate recovered from an anthracnose lesion, was able to cause pepper spot in two seasons. Because fruit that were incubated to obtain anthracnose isolates were visibly free of pepper spot lesions at the time of harvest, it is possible that an isolate from an incipient pepper spot lesion grew actively during the incubation period. As demonstrated in this study, isolates that cause pepper spot are also able to cause anthracnose. Infection by the pepper spot isolate may have taken place in the few days prior to harvest and incubation. In all field inoculation studies, there were no macroscopically visible pepper spot lesions on inoculated fruit 48 h after inoculation when the bags used to maintain high humidity around inoculated fruit were removed, suggesting that at least 2 days are required for pepper spot symptom development.

The isolates that caused pepper spot in this study were very closely related to each other, but not all were identical; ALPS11 and ALAN11 were Genotype 1 and RLPS24 was Genotype 6 (Anderson et al., 2013). Also, isolates ALPS11 and ALPS15 had identical ap-PCR genotypes (Anderson et al., 2013) but only ALPS11 caused pepper spot. In microscopy studies related to this research (Anderson, 2011), it was demonstrated that, when inoculated onto lychee fruit in the field, conidia from both pepper spot and anthracnose isolates were shown to form conidial anastomosis tubes. Such structures would allow for the mechanism for horizontal gene transfer, horizontal chromosome transfer or transmission of a mycovirus to occur. Acquisition of advantageous traits via horizontal transfer could allow for phenotypically distinguishable isolates even if they appear close or even identical by phylogenetic studies, depending on the marker system used. Evidence for such extensive gene transfer within and between fungal species is mounting (Jaramillo et al., 2015; Mehrabi et al., 2011; Mehta & Baghela, 2021). We reiterate our suggestion above that future studies should assess mating compatibility and/or gene flow between different *Colletotrichum* populations to establish the strength of the species boundaries within the gloeosporioides species complex. It would be useful to include genes for virulence factors of *Colletotrichum* spp. such as for pectate lyase (Chudasama et al., 2021; Miyara et al., 2008).

In this study, it was demonstrated that the use of detached fruit assays does not reliably indicate the aggressiveness of an isolate under field conditions. The AUDPC for both the detached fruit assays did not correlate with the severity of anthracnose resulting from inoculation with the same sets of isolates in the field. Studying apple cultivar response rankings to *C. acutatum*, Biggs and Miller (2001) also found poor correlation between field inoculation and detached fruit assays. They speculated the temperature differences between seasons and differences in background inoculum concentrations for early and late season fruit impacted the results for field inoculations. Whilst detached fruit assays are useful for screening cultivars in breeding programmes with well-characterized isolates, caution should be used when applying these assays for assessing the pathogenicity. Many species of *Colletotrichum* exhibit a quiescent stage, commonly causing disease symptoms after harvest and storage (Zakaria, 2021). However, as seen in this work, the length of this quiescent period can vary between genotypes leading to different disease outcomes. Regardless of *Colletotrichum* species being collected from postharvest rots, in phenotyping it is important not to overlook the

potential for them to be preharvest pathogens as well as causing significant damage postharvest.

In the cross-pathogenicity studies, ALPS11 (*C. siamense*) caused large lesions on lychee but caused only small lesions on banana and avocado, and a hypersensitive response on mango. This is in contrast to the isolates that were obtained from lychee but which grouped outside of the tight clade of Genotypes 1 to 9 identified with ap-PCR (Anderson et al., 2013). For example, GLPS12 (*C. fructicola*) and GLPS23 (*C. queenslandicum*) produced moderate anthracnose in field testing but no pepper spot, and in cross-inoculation studies GLPS23 caused smaller lesions on lychee than ALPS11. Both GLPS12 and GLPS23 produced moderate-sized lesions on avocado and appear to not be specialized on lychee, unlike ALPS11.

The inoculation results recorded in the cross-inoculation study were similar to those reported by Hayden et al. (1994) whereby the mango *C. gloeosporioides* isolates produced the largest lesions on mango and *C. musae* the largest lesions on banana, except that in their study the two lychee isolates tested were not as aggressive on lychee relative to their reactions on other fruit. Perhaps both of those isolates were not lychee 'specialists' as neither of the isolates produced very large lesions on detached lychee fruit and the isolates were genetically different from each other. Further pathogenicity testing with more isolates needs to be undertaken on fruit in the field to confirm this host specialization hypothesis.

In the present study using detached fruit assays, definite reactions were recorded on mango, avocado and banana fruit (climacteric fruit), perhaps because fruit were inoculated at the mature but unripe stage and there was still a quiescent (however brief) phase of the pathogen. In this quiescent state, there is an interplay between the host and the pathogen, and defence responses are activated in the host (Prusky et al., 2013). The lychee fruit were inoculated at the mature, ripe stage, a time when entering senescence (Akamine & Goo, 1973) and perhaps are more favourable for the necrotrophic stage of the *Colletotrichum* lifecycle regardless of host preference, again supporting the requirement for field inoculation studies over detached fruit assays.

The present study has demonstrated that specific isolates of *C. siamense* cause pepper spot when inoculated onto lychee fruit, whilst fruit inoculated with other isolates under the same conditions do not develop pepper spot. Whilst environmental conditions, such as those causing host stress, may play a role in pepper spot development, it is clear from this research that there is a genetic basis for the expression of pepper spot disease. There is some evidence of host specialization among isolates of *Colletotrichum* on lychee, which needs to be confirmed with further studies. *C. alienum* and *C. queenslandicum* are reported for the first time as being causal agents of anthracnose of lychee.

ACKNOWLEDGEMENTS

Grower contributions are gratefully acknowledged. Jan Dean, Dr Andrew Miles, Dylan Mazzer and Joy Conroy are thanked for technical assistance. Vivienne Doogan (formerly Department of Primary Industries and Fisheries) provided assistance with statistical analyses. J.A. was a recipient of a Department of Agriculture, Fisheries and Forestry Science and Innovation Award; study leave was provided by Department of Agriculture and Fisheries. All of the anonymous reviewers and Dr Kerry Everett are thanked for their suggestions to improve the manuscript. Open access publishing facilitated by Southern Cross University, as part of the Wiley - Southern Cross University agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Sequence data generated in this study are available from GenBank at https://www.ncbi.nlm.nih.gov/genbank/ with accession numbers provided in Table 2. Other data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Akamine, E.K. & Goo, T. (1973) Respiration and ethylene production during ontogeny of fruit. *Journal of the American Society for Horticultural Science*, 98, 381–383.

Akinsanmi, O.A., Miles, A.K. & Drenth, A. (2007) Timing of fungicide applications for control of husk spot caused by *Pseudocercospora macadamiae* in macadamia. *Plant Disease*, 91, 1675–1681.

Anderson, J.M. (2011) *The diversity of Colletotrichum infecting lychee in Australia*. [PhD thesis]. Brisbane, Australia: The University of Queensland.

Anderson, J.M., Aitken, E.A.B., Dann, E.K. & Coates, L.M. (2013) Morphological and molecular diversity of *Colletotrichum* spp. causing pepper spot and anthracnose of lychee (*Litchi chinensis*) in Australia. *Plant Pathology*, 62, 279–288.

Anderson, J.M. & Coates, L.M. (2009) Lychee. In: Cooke, A.W., Persley, D.M. & House, S. (Eds.) *Diseases of fruit crops in Australia*. Collingwood: CSIRO Publishing, pp. 145–148.

Biggs, A.R. & Miller, S.S. (2001) Relative susceptibility of selected apple cultivars to *Colletotrichum acutatum*. *Plant Disease*, 85, 657–660.

Buchfink, B., Xie, C. & Huson, D.H. (2015) Fast and sensitive protein alignment using DIAMOND. *Nature Methods*, 12, 59–60.

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17, 540–552.

Chudasama, K.S., Monpara, J.K. & Thaker, V.S. (2021) Identification and characterization of pectin lyase gene as a virulence factor in *Colletotrichum gloeosporioides*. *Physiological and Molecular Plant Pathology*, 116, 101706.

Coates, L.M., Zhou, E. & Sittigul, C. (2005) Diseases. In: Menzel, C.M. & Waite, G.K. (Eds.) *Litchi and longan: botany, production and uses*. Wallingford: CABI Publishing, pp. 261–272.

Cooke, A.W. & Coates, L.M. (2002) Pepper spot: a preharvest disease of lychee caused by *Colletotrichum gloeosporioides*. *Australasian Plant Pathology*, 31, 303–304.

De Silva, D.D., Ades, P.K., Crous, P.W. & Taylor, P.W.J. (2017) *Colletotrichum* species associated with chili anthracnose in Australia. *Plant Pathology*, 66, 254–267.

Diczbalis, Y. (2012) *Tropical exotic fruit industry: strategic direction setting 2012–2015, publication no. 12/050*. Canberra: Rural Industries Research and Development Corporation.

Drew, H. & Drew, J. (2001) *Lychee pepper spot in Australia (its impact and control)*. Canberra: Rural Industries Research and Development Corporation.

Emms, D.M. & Kelly, S. (2019) OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology*, 20, 238.

Fitzell, R.D. (1981) Effects of regular applications of benomyl on the population of *Colletotrichum* in mango leaves. *Transactions of the British Mycological Society*, 77, 529–533.

Fitzell, R.D. & Coates, L.M. (1995) Lychees. In: Coates, L.M., Cooke, A.W., Persley, D., Beattie, B.B., Wade, N.L. & Ridgway, R. (Eds.) *Postharvest diseases of horticultural produce. Tropical fruit*. Brisbane: Queensland Department of Primary Industries, pp. 41–45.

Giblin, F.R. (2006) *Avocado fruit responses to C. gloeosporioides*. [PhD thesis]. Brisbane, Australia: University of Queensland.

Giblin, F.R., Coates, L.M. & Irwin, J.A.G. (2010) Pathogenic diversity of avocado and mango isolates of *Colletotrichum gloeosporioides* causing anthracnose and pepper spot in Australia. *Australasian Plant Pathology*, 39, 50–62.

Giblin, F.R., Tan, Y.P., Mitchell, R., Coates, L.M., Irwin, J.A.G. & Shivas, R.G. (2018) *Colletotrichum* species associated with pre-and postharvest diseases of avocado and mango in eastern Australia. *Australasian Plant Pathology*, 47, 269–276.

Hayden, H.L., Pegg, K.G., Aitken, E.A.B. & Irwin, J.A.G. (1994) Genetic relationships as assessed by molecular markers and cross-infection among strains of *Colletotrichum gloeosporioides*. *Australian Journal of Botany*, 42, 9–18.

Hort Innovation. (2022) Australian Horticulture Statistics Handbook 2020/2021. Available from: https://www.horticulture.com.au/contentassets/a68c8934a8bf40b4becdc487bacdb60f/hort-innovation-ahsh-20-21-fruit.pdf [Accessed 15th March 2024].

Huson, D.H. & Kloepper, T.H. (2005) Computing recombination networks from binary sequences. *Bioinformatics*, 21(Suppl 2), ii159–ii165.

Jaramillo, V.D.A., Sukno, S.A. & Thon, M.R. (2015) Identification of horizontally transferred genes in the genus *Colletotrichum* reveals a steady tempo of bacterial to fungal gene transfer. *BMC Genomics*, 16, 2.

Johnson, G.I., Cooke, A.W. & Sardsud, U. (2002) Postharvest disease control in lychee. *Acta Horticulturae*, 575, 705–715.

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.

Kück, P. & Longo, G.C. (2014) FASconCAT-G: extensive functions for multiple sequence alignment preparations concerning phylogenetic studies. *Frontiers in Zoology*, 11, 81.

Li, S., Zhao, Y., Wang, X., Hu, F., Chen, Z. & Fan, H. (2021) Hǎinán lìzhī tànjū bìng bìngyuánjùn jiàndìng jí yíchuán duōyàng xìng fēnxī [Identification and genetic diversity analysis of *Litchi chinensis Colletotrichum* spp.in Hainan]. *Journal of Agricultural Biotechnology*, 29, 673–687.

Ling, J.F., Song, X.B., Xi, P.G., Cheng, B.P., Cui, Y.P., Chen, X. et al. (2019) Identification of *Colletotrichum siamense* causing litchi pepper spot disease in mainland China. *Plant Pathology*, 68, 1533–1542.

Mehrabi, R., Bahkali, A.H., Abd-Elsalam, K.A., Moselm, M., Ben M'barek, S., Gohari, A.M. et al. (2011) Horizontal gene and chromosome transfer in plant pathogenic fungi affecting host range. *FEMS Microbiology Reviews*, 35, 542–554.

Mehta, N. & Baghela, A. (2021) Quorum sensing-mediated inter-specific conidial anastomosis tube fusion between *Colletotrichum gloeosporioides* and *C. siamense*. *IMA Fungus*, 12, 7.

Menzel, C. & McConchie, C. (1998) Lychee and longan. In: Hyde, K. (Ed.) *The new rural industries. A handbook for farmers and investors*. Canberra: Rural Industries Research and Development Corporation, pp. 288–295.

Minh, B.Q., Hahn, M.W. & Lanfear, R. (2020) New methods to calculate concordance factors for phylogenomic datasets. *Molecular Biology and Evolution*, 37, 2727–2733.

Minh, B.Q., Nguyen, M.A.T. & von Haeseler, A. (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, 30, 1188–1195.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. et al. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37, 1530–1534.

Miyara, I., Shafran, H., Kramer Haimovich, H., Rollins, J., Sherman, A. & Prusky, D. (2008) Multi-factor regulation of pectate lyase secretion by *Colletotrichum gloeosporioides* pathogenic on avocado fruits. *Molecular Plant Pathology*, 9, 281–291.

Nakasone, H.Y. & Paull, R.E. (1998) *Tropical fruits*. Wallingford: CAB International.

Newett, S., Whiley, A.W., Dirou, J., Hofman, P., Ireland, G., Kernot, I. et al. (2001) *Avocado information kit*. Nambour: Queensland Department of Primary Industries.

Ni, H., Huang, C., Wu, C., Yang, H., Lin, C.Y., Chang, J. et al. (2017) First report of pepper spot disease of lychee caused by *Colletotrichum siamense* in Taiwan. *Journal of Plant Pathology*, 99, 808.

Palmer, J. & Stajich, J.E. (2019) nextgenusfs/funannotate: funannotate v1.5.3. Available from: https://zenodo.org/records/2604804 [Accessed 15th March 2024].

Pegg, K.G., Coates, L.M., Korsten, L. & Harding, R.M. (2002) Foliar, fruit and soilborne diseases. In: Whiley, A.W., Schaffer, B. & Wolstenholme, B.N. (Eds.) *The avocado: botany, production and uses*. Wallingford: CABI Publishing, pp. 299–338.

Prusky, D., Alkan, N., Fluhr, R. & Tesfaye, M. (2013) Quiescent and necrotrophic lifestyle choice during postharvest disease development. *Annual Review of Phytopathology*, 51, 155–176.

Schoeman, M.H. & Manicom, B.Q. (2000) Report on the epidemiology of pepper spot on 'Hass' avocado. *South African Avocado Growers' Association Yearbook*, 23, 95–97.

Scott, K.J., Brown, B.I., Chaplin, G.R., Wlicox, M.E. & Bain, J.M. (1982) The control of rotting and browning of litchi fruit by hot benomyl and plastic film. *Scientia Horticulturae*, 16, 253–262.

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, 117–138.

Sharma, G., 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, 15839.

Shivas, R.G. & Tan, Y.P. (2009) A taxonomic re-assessment of Colletotrichum acutatum, introducing C. fioriniae comb. et stat. nov. and C. simmondsii sp. nov. *Fungal Diversity*, 39, 111–122.

Shivas, R.G., Tan, Y.P., Edwards, J., Dinh, Q., Maxwell, A., Andjic, V. et al. (2016) *Colletotrichum* species in Australia. *Australasian Plant Pathology*, 45, 447–464.

Taylor, J.W., Hann-Soden, C., Branco, S., Sylvain, I. & Ellison, C.E. (2015) Clonal reproduction in fungi. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 8901–8908.

Tibayrenc, M. & Ayala, F.J. (2012) Reproductive clonality of pathogens: a perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. *Proceedings of the National Academy of Sciences of the United States of America*, 109, E3305–E3313.

Wang, W., de Silva, D.D., Moslemi, A., Edwards, J., Ades, P.K., Crous, P.W. et al. (2021) *Colletotrichum* species causing anthracnose of citrus in Australia. *Journal of Fungi*, 7, 47.

Weir, B.S., Johnston, P.R. & Damm, U. (2012) The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology*, 73, 115–180.

Willingham, S.L., Cooke, A.W., Coates, L.M. & Pegg, K.G. (2000) Pepper spot: a new preharvest *Colletotrichum* disease of avocado cv. Hass. *Australasian Plant Pathology*, 29, 151.

Zakaria, L. (2021) Diversity of *Colletotrichum* species associated with anthracnose disease in tropical fruit crops—a review. *Agriculture*, 30, 297.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Anderson, J.M., Coates, L.M., Aitken, E.A.B., Mitchell, R., McTaggart, A.R. & Dann, E.K. (2024) The pathogenic diversity and host range of *Colletotrichum* spp. causing pepper spot and anthracnose of lychee (*Litchi chinensis*) in Australia. *Plant Pathology*, 00, 1–15. Available from: https://doi.org/10.1111/ppa.13901