Colletotrichum species associated with loquat anthracnose in Kagawa and Tokushima prefectures, Japan

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Research Article

Abstract

Anthracnose caused by *Colletotrichum* species is one of the serious diseases of loquat, but diversification of the species of *Colletotrichum* associated with loquat in Japan has not been adequately surveyed. In this study, 46 isolates were isolated from diseased leaves and fruits of loquat in Kagawa and Tokushima prefectures in 2017 and 2018. Using a combination of morphological features and molecular approaches, seven *Colletotrichum* species were identified: *C. fioriniae*, *C. nymphaeae*, *C. karsti*, *C. aenigma*, *C. fructicola*, *C. gloeosporioides* s. s., and *C. siamense*. The most prevalent species was *C. karsti* followed by *C. fioriniae* and *C. gloeosporioides* s. s., accounting for 43.5, 21.7, and 13.0%, respectively. All identified species were confirmed to be the causal agents of loquat anthracnose by applying Koch's postulates. Moreover, potential cross-infection from loquat to kiwifruit, satsuma mandarin, persimmon, and peach was found, in which loquat isolates of *C. fioriniae*, *C. fructicola*, and *C. siamense* caused symptoms on all the tested plants. This report is the first on *C. karsti* and *C. aenigma* associated with loquat anthracnose and the first record of *C. fructicola* and *C. gloeosporioides* s. s. in Japan, providing crucial information for epidemiology.

Introduction

Loquat (*Eriobotrya japonica* (Thunb.) Lindl) is an ancient subtropical evergreen fruit tree originating in China (Tian et al. 2011). Loquat fruit is rich in essential dietary nutrients including sugars, protein, carotenoids, fiber, vitamins A and C, and minerals (Tian et al. 2011; Li et al. 2016). Loquat leaves are rich in fiber, minerals, and vitamins B2, B6, and B12, and have several medicinal properties such as antioxidant, antidiabetic, antitumoral, anti-inflammatory, antinociceptive, and antimicrobial (Cha et al. 2011; Khouya et al. 2022; Kuraoka-Oliveira et al. 2020; Shen et al. 2021). These properties lead loquat to be commercially cultivated in more than 20 countries including China, India, Australia, Brazil, Italy, Spain, Turkey, and Japan (Tian et al. 2007, 2011). In Japan, loquat is a minor crop with about 2,500 tons harvested in 2022 from 905 ha (Ministry of Agriculture, Forestry and Fisheries; https://www.maff.go.jp/index.html). Nevertheless, the appearance of foliar and fruit diseases has been found annually in loquat cultivation, with little attention being paid to disease management.

More than 200 fungal species have been associated with loquat worldwide (Farr and Rossman 2023). Among them, anthracnose caused by *Colletotrichum* species is one of the serious diseases that damages fruit quality and is considered the major postharvest disease of loquat fruit (Cao et al. 2008; Palou et al. 2016). Infected fruits show brown sunken lesions, which later turn into black, hard, and shriveled mummies (Naz et al. 2017). The fungal pathogen can infect leaves, which causes small brown to reddish-brown necrotic spots or sunken lesions (Naz et al. 2017; Kuang et al. 2021). Under high humidity, the whole fruit may rot, and fungal fruiting bodies with pale orange to orange spore masses form on the rotten surface (Cao et al. 2008; Cao and Zheng 2010; Liu et al. 2007).

The genus *Colletotrichum* was first introduced by Corda (1831) and is the sole member of the family Glomerellaceae (Glomerellales, Sordariomycetes) (Maharachchikumbura et al. 2016; Hyde et al. 2016, 2020). This genus comprises more than 248 accepted species belonging to 14 species complexes and 13 singletons (Jayawardena et al. 2021). Many are important phytopathogens, and some are endophytes and saprobes (Cannon et al. 2012; Hyde et al. 2014, 2020; Jayawardena et al. 2021). In loquat, seven species of *Colletotrichum* have been recorded including *C. fioriniae*, *C. nymphaeae*, *C. godetia*, and *C. eriobotryae* from the *C. acutatum* species complex (CASC) and *C. fructicola*, *C. gloeosporioides* s. s., and *C. siamense* from the *C. gloeosporioides* species complex (CGSC) (Damm et al. 2020; Farr and Rossman 2023; James et al. 2014; Juárez-Vázquez et al. 2019; Kuang et al. 2021; Naz et al. 2017; Sato et al. 2013). However, only *C. fioriniae*, *C. nymphaeae*, and *Colletotrichum* sp. have been recorded in Japan, and these species were last isolated more than a decade ago (NARO Genebank, https://www.gene.affrc.go.jp/databases-micro_search_en.php?pldis=5601). Therefore, the species diversification of *Colletotrichum* in Japan may have changed.

In Kagawa Prefecture, Japan, loquat is cultivated in orchards, in-house, by the roadside, and near other important economic fruit crops such as kiwifruit, satsuma mandarin, persimmon, and peach. According to several reports, some *Colletotrichum* species have a wide host range, and the phenomenon of cross-infection from the original host to other hosts has been reported (de Aguiar Carraro et al. 2022; Eaton et al. 2021; Jayawardena et al. 2016; Moral et al. 2021; Talhinhas and Baroncelli 2021). This finding leads to a concern that cross-infection by *Colletotrichum* species might occur between loquat and other proximately cultivated fruit crops. If cross-infection does occur, loquat might be an inoculum source and an alternate host for the overwintering of pathogens, indicating the necessity to pay attention to disease management.

Therefore, this study aimed to identify the *Colletotrichum* species associated with loquat in Kagawa and Tokushima prefectures based on morphological characteristics and phylogenetic analysis and evaluate the potential for cross-infection by isolates from loquat to

kiwifruit, satsuma mandarin, persimmon, and peach.

Materials and methods

Sample collection, fungal isolation, and morphological examination

Diseased fruits and leaves of loquat were collected from orchards in Kagawa and Tokushima prefectures, Japan, in 2017 and 2018. A segment (5 × 5 mm) was cut from the boundary of diseased and healthy tissue using a sterilized scalpel. The tissue was surface sterilized by immersion in 1% sodium hypochlorite for 1 min followed by rinsing three times in sterilized distilled water to remove the sterilizing reagent. After drying by blotting with a sterilized paper towel, the tissue was placed in the center of a Petri dish containing potato dextrose agar (PDA; Nissui Pharmaceutical, Tokyo, Japan), which was then incubated at 25°C for 3–5 days in the dark. The hyphal tips cut from mycelia growing from the tissue were transferred to a new Petri dish containing PDA and incubated at 25°C for 7–14 days in the dark. Conidia were harvested to prepare a conidial suspension for purifying the isolate by single spore isolation. Cultural and morphological characterization was examined following the method described previously (Cai et al. 2009; Yu et al. 2022). For long-term storage, a conidia suspension in 25% glycerol of individual pure culture was maintained at − 80°C.

DNA extraction, PCR amplification, and sequencing

The genomic DNA of the isolate was extracted using the CTAB extraction method with slight modifications (Lee et al. 1988; Zhang et al. 2010) and finally eluted with nucleic acid-free water. The DNA was quantified using a NanoDrop1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the concentration adjusted to 50 ng/µl to use as the template for PCR reactions. The sequence of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene of all the isolates was amplified and sequenced using primers GDF1 and GDR1 (Templeton et al. 1992). Furthermore, representative isolates were selected to amplify the sequences of the internal transcribed spacer (ITS) region of rDNA, ß-tubulin (*TUB2*), actin (*ACT*), chitin synthase 1 (*CHS-1*), and calmodulin (*CAL*, for the species belonging to CGSC and CBSC) genes using primer pairs ITS1/ITS4 (White et al. 1990), T1/Bt2b (O'Donnell and Cigelnik 1997; Glass and Donaldson 1995), ACT512F/ACT783R, CHS-79F/CHS-345R (Carbone and Kohn 1999), and CL1C/CL2C (Weir et al. 2012), respectively. PCR was performed under the conditions described by Poti et al. (2023). The PCR products were purified using the PCR clean-up Gel extraction kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and sequenced in both forward and reverse directions by Fasmac Co. Ltd. (Kanagawa, Japan). The consensus sequences generated in this study were deposited in GenBank (Table 1).

Table 1
GenBank accession numbers of the *Colletotrichum* isolates generated in this study

Species	Isolate	Host	GenBank accession number[a]					
			GAPDH	ITS	*TUB2*	*ACT*	*CHS-1*	*CAL*
Colletotrichum fioriniae	KMI-2-2-Ca1	*Eriobotrya japonica* cv. Mogi	OQ440351	OQ430706	OQ440361	OQ440324	OQ440341	-
	KMI-2-2-Ca2	*E. japonica* cv. Mogi	OQ440352	OQ430707	OQ440362	OQ440325	OQ440342	-
C. nymphaeae	TSK-1-Cg2	*E. japonica* cv. Nagasakiwase	OQ440358	OQ430713	OQ440368	OQ440331	OQ440348	-
C. karsti	YMZ-2-1-Cg1	*E. japonica*	OQ440349	OQ430704	OQ440359	OQ440322	OQ440339	OQ440332
	NB-2-2-Cg1	*E. japonica*	OQ440350	OQ430705	OQ440360	OQ440323	OQ440340	OQ440333
	TI-1-1-2-Cg2	*E. japonica* cv. Mogi	OQ440356	OQ430711	OQ440366	OQ440329	OQ440346	OQ440337
C. aenigma	STO-2-2-2-Cg2	*E. japonica* cv. Mogi	OQ440357	OQ430712	OQ440367	OQ440330	OQ440347	OQ440338
C. fructicola	ZYH-3-1-2-Ca1	*E. japonica*	OQ440354	OQ430709	OQ440364	OQ440327	OQ440344	OQ440335
C. gloeosporioides s. s.	ZYH-1-3-2-Cg1	*E. japonica* cv. Mogi	OQ440353	OQ430708	OQ440363	OQ440326	OQ440343	OQ440334
C. siamense	FuchuS-3-Cg2	*E. japonica* cv. Mogi	OQ440355	OQ430710	OQ440365	OQ440328	OQ440345	OQ440336

[a] *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase, ITS = internal transcribed spacer region of ribosomal DNA, *TUB2* = β-tubulin, *ACT* = actin, *CHS-1* = chitin synthase 1, and *CAL* = calmodulin

Phylogenetic analysis

Multilocus phylogenetic analysis based on Bayesian inference (BI) was separately conducted for the three species complexes. Five loci (ITS, *GAPDH*, *CHS-1*, *ACT*, and *TUB2*) were used to generate a concatenated dataset for the *C. acutatum* species complex (Damm et al. 2012a); six loci (ITS, *GAPDH*, *CHS-1*, *ACT*, *TUB2*, and *CAL*) were used for the *C. boninense* species complex (Damm et al. 2012b); and six loci (*ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS) were used for the *C. gloeosporioides* species complex (Jayawardena et al. 2016). The sequences of type isolates of each species used for the analyses were gained from GenBank and are listed in Supplementary Tables S1 to S3. Outgroups for the *C. acutatum*, *C. boninense*, and *C. gloeosporioides* species complex analyses were *Colletotrichum orchidophilum* Allesch. (CBS 632.80), *C. gloeosporioides* s. s. (CBS 112999), and *C. boninense* Moriwaki, Toy. Sato & Tsukib. (CBS 123755), respectively. Multiple sequences were aligned using MAFFT v.7 (Katoh et al. 2019), combined, and manually improved where necessary using BioEdit v.7 (Informer Technologies, Inc., California, USA). BI analysis was carried out through the CIPRES Science Gateway v.3.3 (Miller et al. 2010; https://www.phylo.org) with the parameters described by Tan et al. (2022). The phylogenetic trees were illustrated using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator 2022 (Adobe Inc., San Jose, California, USA).

Pathogenicity test

The representative isolate of each *Colletotrichum* sp. was used in pathogenicity tests on detached fruits of the loquat cultivar 'Mogi'. Moreover, detached fruits of persimmon (*Diospyros kaki* cultivar 'Fuyu'), kiwifruit (*Actinidia chinensis* cultivar 'Sanuki Angle Sweet'), peach (*Prunus persica* cultivar 'Hashiba-hakuho'), and satsuma mandarin (*Citrus unshiu* cultivar 'Aoshima Unshu') were used to assess the potential for cross-infection. The healthy fruits of these plants were harvested from experimental fields of the Fuchu Fruit Research Institute, Kagawa, Japan. The fruits were rinsed with sterilized distilled water several times and air-dried. Two inoculation sites (intact and wounded) were set for each fruit, and three fruits of each plant were used per isolate. To create a wound, a sterilized needle was

inserted into the fruits to a depth of 3 mm. A mycelial plug cut from the margin of a 7-day-old colony was placed upside down on the inoculation site, while control fruits were inoculated with a PDA plug. The inoculated fruits were placed in a moist plastic box and incubated at 25°C for 7 days. Symptoms were recorded every day and photographed at 7 days of inoculation. The experiment was conducted twice.

Results

Fungal isolation and morphological characterization

A total of 46 isolates were isolated from the symptomatic leaves and fruits of loquat in Kagawa and Tokushima prefectures, Japan, of which 40 were from the leaves and 6 were from the fruits. Based on culture and conidial characteristics, the isolates were classified into 8 morphotypes (Fig. 1, Table 2). The highest number of isolates found were morphotype III with 11 isolates followed by morphotypes I, IV, VII, and VIII with 10, 9, 6, and 4 isolates, respectively. The least common were morphotypes II, V, and VI with only 2 isolates each. The isolates with morphotypes I and II producing fusiform conidia with both ends acute were assigned to the *Colletotrichum acutatum* species complex (CASC), while isolates with morphotypes III and IV producing cylindrical conidia with a prominent hilum were assigned to the *C. boninense* species complex. The isolates with morphotypes V to VIII producing cylindrical conidia were assigned to the *C. gloeosporioides* species complex.

Table 2

Morphological characteristics of the *Colletotrichum* species causing kiwifruit anthracnose in Japan

Morphotype	Isolate name[a]	Colony character on PDA	Conidia	Appressoria
Type I (*C. fioriniae*)	**KMI-2-2-Ca1** **KMI-2-2-Ca2** FuchuB-1-1-Ca1 FuchuB-1-1-Ca2 Yuta1-Ca1 Yuta1-Ca2 Yuta4-Ca1 Yuta4-Ca2 Yuta7-1-Ca1 Yama4-3-Ca1	Cottony, reddish colony with white to olivaceous gray aerial mycelia dense at the center. Reverse: reddish. Orange conidial masses were observed to be randomly distributed but dense at the center. Growth rate = 8.8 and 8.7 mm/day	Hyaline, smooth-walled, aseptate, straight, ellipsoid to fusiform, both ends acute with size of $13.0 \pm 0.9 \times 4.5 \pm 0.3$ µm (L/W ratio = 2.9) or $12.9 \pm 1.0 \times 4.6 \pm 0.4$ µm (L/W ratio = 2.8)	Sepia to medium brown, smooth-walled, globose to ovate, $16.6 \pm 2.5 \times 12.1 \pm 1.5$ µm (L/W ratio = 0.7) or $9.0 \pm 1.2 \times 6.8 \pm 0.9$ µm (L/W ratio = 0.8)
Type II (*C. nymphaeae*)	TSK-1-Cg1 **TSK-1-Cg2**	White to light pale gray aerial mycelia dense at the center. Reverse: pale orange to olivaceous gray. Orange conidial masses were observed at the center of the colony. Growth rate = 9.2 mm/day	Hyaline, smooth-walled, aseptate, straight, ellipsoid to fusiform, both ends sharp with size of $14.8 \pm 1.8 \times 4.7 \pm 0.8$ µm (L/W ratio = 3.1)	Medium brown, smooth-walled, globose to ovate, $10.9 \pm 2.3 \times 7.3 \pm 1.1$ µm (L/W ratio = 0.7)

[a] Isolate name in bold was selected as representative isolate for describing morphological characteristics and for phylogenetic analyses.

Morphotype	Isolate name[a]	Colony character on PDA	Conidia	Appressoria
Type III (*C. karsti*)	**YMZ-2-1-Cg1** **NB-2-2-Cg1** ZYH-1-1-2-Cg1 ZYH-1-1-2-Cg2 ZYH-2-3-Cg2 ZYH-3-1-1-Cg1 ZYH-3-1-1-Cg2 ZYH-3-2-2-Cg1 ZYH-3-2-2-Cg2 Yuta7-1-Cg1 Yuta7-1-Cg2	White aerial mycelium. Reverse: pale yellow. Growth rate = 9.0 and 9.7 mm/day	Hyaline, smooth-walled, aseptate, straight, cylindrical with rounded ends and a hilum-like base, $14.3 \pm 0.9 \times 5.9 \pm 0.4$ µm (L/W ratio = 2.4) or $13.5 \pm 1.1 \times 6.2 \pm 0.7$ µm (L/W ratio = 2.2)	Sepia to medium brown, smooth-walled, sometimes lobed, globose to ovate, $8.8 \pm 1.3 \times 6.5 \pm 0.9$ µm (L/W ratio = 0.7) or $8.8 \pm 1.3 \times 6.2 \pm 0.9$ µm (L/W ratio = 0.7)
Type IV (*C. karsti*)	YMZ-2-1-Cg3 NB-2-2-Cg2 ZYH-2-3-Cg1 TI-1-1-1-Cg1 TI-1-1-1-Cg2 TI-1-1-2-Cg1 **TI-1-1-2-Cg2** Tob-2-2-1-Cg1 Tob-2-2-1-Cg2	Thin white aerial mycelium with randomly distributed orange spore masses. Reverse: light yellow with orange spots of conidial masses. Growth rate = 10.2 mm/day	Hyaline, smooth-walled, aseptate, straight, cylindrical, rounded ends with a hilum-like base, $14.9 \pm 0.9 \times 6.7 \pm 0.7$ µm (L/W ratio = 2.2)	Sepia to medium brown, smooth-walled, sometimes lobed, globose to ovate, $8.1 \pm 0.9 \times 6.4 \pm 0.6$ µm (L/W ratio = 0.8)
Type V (*C. aenigma*)	STO-2-2-2-Cg1 **STO-2-2-2-Cg2**	Olivaceous gray colony with flat white to pale gray aerial mycelia. Reverse: Olivaceous gray to brown. Growth rate = 12.2 mm/day (calculated at 5 d)	Hyaline, smooth-walled, aseptate, straight or slightly distorted, cylindrical with one slightly acute or broadly rounded ends, $14.2 \pm 1.5 \times 4.1 \pm 0.5$ µm (L/W ratio = 3.5)	Sepia to medium brown, irregular, lobed, $9.5 \pm 1.4 \times 7.1 \pm 0.7$ µm (L/W ratio = 0.8)

[a] Isolate name in bold was selected as representative isolate for describing morphological characteristics and for phylogenetic analyses.

Morphotype	Isolate name[a]	Colony character on PDA	Conidia	Appressoria
Type VI (*C. fructicola*)	**ZYH-3-1-2-Ca1**	Olivaceous gray colony with pale gray aerial mycelia. Reverse: olivaceous gray to dark gray, dark at the center of colony. Growth rate = 12.2 mm/day (calculated at 5 d)	Hyaline, smooth-walled, aseptate, cylindrical with rounded ends, 13.0 ± 1.0 × 4.3 ± 0.5 μm (L/W ratio = 3.0)	Sepia to medium brown, smooth-walled, ovate,
	ZYH-3-1-2-Ca2			9.4 ± 1.6 × 7.4 ± 1.2 μm (L/W ratio = 0.8)
Type VII (*C. gloeosporioides* s. s.)	**ZYH-1-3-2-Cg1**	Cottony, white to pale gray aerial mycelia. Reverse: olivaceous gray, dark at the center. Growth rate = 10.2 mm/day	Hyaline, smooth-walled, aseptate, straight cylindrical with rounded ends, 14.4 ± 1.0 × 4.6 ± 0.6 μm (L/W ratio = 3.2)	Sepia to medium brown, smooth-walled, ovate to clavate, 8.6 ± 1.4 × 6.2 ± 0.7 μm (L/W ratio = 0.7)
	ZYH-1-3-2-Cg2			
	ZYH-3-3-2-1-Ca1			
	ZYH-3-3-2-1-Ca2			
	Yuta3-1-Cg1			
	Yuta3-1-Cg2			
Type VIII (*C. siamense*)	FuchuS-3-Cg1	Cottony, white aerial mycelia and white on the reverse side. Growth rate = 11.6 mm/day	Hyaline, smooth-walled, aseptate, cylindrical with rounded ends, 13.4 ± 0.8 × 4.9 ± 0.5 μm (L/W ratio = 2.7)	Sepia to medium brown, smooth-walled, ovate, 9.1 ± 1.4 × 6.8 ± 0.8 μm (L/W ratio = 0.8)
	FuchuS-3-Cg2			
	TI-1-2-Ca1			
	TI-1-2-Ca2			

[a] Isolate name in bold was selected as representative isolate for describing morphological characteristics and for phylogenetic analyses.

Phylogenetic analysis

Preliminary analysis of the *GAPDH* sequences of all the isolates using a BLAST search in the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov) showed that the isolates with morphotypes I to VIII were similar to *Colletotrichum fioriniae* R.G. Shivas & Y.P. Tan, *C. nymphaeae* (Pass.) Aa, *C. karsti* You L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, *C. karsti*, *C. aenigma* B.S. Weir & P.R. Johnst., *C. fructicola* Prihast., L. Cai & K.D. Hyde, *C. gloeosporioides* (Penz.) Penz. & Sacc. sensu stricto, and *C. siamense* Prihast., L. Cai & K.D. Hyde, respectively. To confirm the results, multiple loci phylogenetic analyses of the representative isolates from each morphotype were conducted. The phylogram of the CASC shows isolates KMI-2-2-Ca1 and KMI-2-2-Ca2 (morphotype I) are clustered with *C. fioriniae* (CBS 127601 and CBS 119186), and isolate TSK-1-Cg1 (morphotype II) is clustered with *C. nymphaeae* (CBS 125996, CBS 231.49, and CBS 515.78), with Bayesian posterior probabilities of 1 for all (Fig. 2a). The CBSC phylogram shows isolates YMZ-2-1-Cg1, NB-2-2-Cg1 (morphotype III), and TI-1-1-2-Cg2 (morphotype IV) are clustered with *C. karsti* (Bayesian posterior probabilities = 1) (Fig. 2b). The CGSC phylogram shows isolates STO-2-2-2-Cg2 (morphotype V), ZYH-3-1-2-Ca1 (morphotype VI), ZYH-1-3-2-Cg1 (morphotype VII), and FuchuS-3-Cg2 (morphotype VIII) are clustered with *C. aenigma*, *C. fructicola*, *C. gloeosporioides*, and *C. siamense*, respectively, with Bayesian posterior probabilities of 1 for all (Fig. 2c). These results confirmed that seven species of *Colletotrichum* were isolated from loquat, of which *C. karsti* was the most prevalent in the population (20 isolates).

Pathogenicity on loquat fruit and cross-infection on the other fruits

A pathogenicity test on the loquat fruits showed that all the isolates caused anthracnose symptoms at the wounded site. Only *C. nymphaeae* (TSK-1-Cg2), *C. fructicola* (ZYH-1-3-2-Ca1), and *C. siamense* (FuchuS-3-Cg2) produced symptoms at both the wounded and intact sites; however, the lesions on the wounded sites were larger than on the intact sites (Fig. 3, Table 3). The symptoms were brown sunken necrotic lesions, and the surrounding tissue was abnormally soft. Symptoms caused by the different isolates were indistinguishable, except the fruit inoculated with *C. fructicola* had dark, visible fruiting bodies. No symptoms appeared on the control fruits. Moreover, the fungus could be successfully re-isolated from the inoculated fruits and had morphological features similar to the original isolate. These results satisfied Koch's postulates and verified that all seven species of *Colletotrichum* identified in this study are the causal agent of loquat anthracnose.

Table 3
Pathogenicity test of the representative isolates of *Colletotrichum* on loquat, kiwifruit, satsuma mandarin, persimmon, and peach fruits.

Species	Isolate	Loquat[a]		Kiwifruit		satsuma mandarin		Persimmon		Peach	
		W[b]	I	W	I	W	I	W	I	W	I
C. fioriniae	KMI-2-2-Ca2	++	−	++	−	+	−	+	+	+	+
C. nymphaeae	TSK-1-Cg2	++	+	+	−	+	−	+	−	−	−
C. karsti	YMZ-2-1-Cg1	++	−	+	−	−	−	+	−	−	−
C. aenigma	STO-2-2-2-Cg2	++	−	+	−	−	−	+	−	−	−
C. fructicola	ZYH-3-1-2-Ca1	++	+	++	−	+	−	+	+	++	+
C. gloeosporioides s. s.	ZYH-1-3-2-Cg1	++	−	+	−	−	−	+	−	−	−
C. siamense	FuchuS-3-Cg2	++	+	++	−	+	−	+	−	++	++

[a] − indicates no symptom developed, + indicates a small lesion, ++ indicates a lesion larger than the inoculation site. [b] W and I are wounded and intact sites, respectively

Inoculation tests on the fruits of kiwifruit, satsuma mandarin, persimmon, and peach were conducted. The results showed that *C. fioriniae* (YMZ-2-1-Cg1) caused the symptoms on all the tested plants, and severe symptoms were observed on kiwifruit at the wounded sites (Fig. 4, Table 3). *Colletotrichum fructicola* (ZYH-1-3-2-Ca1) and *C. siamense* (FuchuS-3-Cg2) also produced symptoms on all the tested plants, but severe symptoms were observed on kiwifruit and peach at the wounded sites. *Colletotrichum karsti* (YMZ-2-1-Cg1), *C. aenigma* (STO-2-2-2-Cg2), and *C. gloeosporioides* s. s. (ZYH-1-3-2-Cg1) caused mild symptoms on kiwifruit and persimmon at the wounded sites, and *C. nymphaeae* (TSK-1-Cg2) caused mild symptoms on kiwifruit, satsuma mandarin, and persimmon at the wounded sites. These results indicated that all seven *Colletotrichum* species have a wide host range, with *C. fructicola* and *C. siamense* appearing to be more aggressive than the other species.

Discussion

In the present study, seven *Colletotrichum* species from three species complexes were identified in loquat in Japan based on a combination of their morphological characteristics and multiple gene phylogenetic analysis: *C. fioriniae* and *C. nymphaeae* from the *C. acutatum* species complex; *C. karsti* from the *C. boninense* species complex; and *C. aenigma*, *C. fructicola*, *C. gloeosporioides* s. s., and *C. siamense* from the *C. gloeosporioides* species complex. Of the seven species, *C. fioriniae*, *C. nymphaeae*, *C. fructicola*, *C. gloeosporioides* s. s., and *C. siamense* have been reported to be associated with loquat (Damm et al. 2020; Farr and Rossman 2023; James et al. 2014; Juárez-Vázquez et al. 2019; Kuang et al. 2021; Naz et al. 2017; Sato et al. 2013). Only *C. fioriniae*, *C. nymphaeae*, and *Colletotrichum* sp. (MAFF 424604) have been recorded in Japan (https://www.gene.affrc.go.jp/databases-micro_search_en.php?pldis=5601). Careful checking of the *ACT*, *GAPDH*, *TUB2*, and ITS sequences of *Colletotrichum* sp. (MAFF 242604) revealed that all the sequences were similar to *C. siamense*. Moreover, the *GAPDH* and *TUB2* sequences, which are crucial DNA barcodes (Cannon et al. 2012), were identical to our identified *C. siamense* (FuchuS-1-Cg1). Therefore, we suspected *Colletotrichum* sp. (MAFF 242604) was *C. siamense*, which led us to conclude that the three species *C. fioriniae*, *C. nymphaeae*, and *C. siamense* were previously found in Japan. Nevertheless, the finding in the present study is the first report of *C. karsti* and *C. aenigma* associated with loquat anthracnose and the first record of *C. fructicola* and C. *gloeosporioides* s. s. in Japan.

A pathogenicity test on the loquat fruits verified that all species produced the symptoms at the wounded sites, and *C. nymphaeae*, *C. fructicola*, and *C. siamense* produced slight symptoms at the intact sites. This finding indicated that wounding is conducive to the pathogenicity of these isolates. Several previous studies also showed that wounding increases the infectiousness and virulence of *Colletotrichum* spp. (de Aguiar Carraro et al. 2022; Guo et al. 2022; Jiang et al. 2014; Silva et al. 2020; Tan et al. 2023). Moreover, a pathogenicity test on the other plants showed that the isolates generally have the potential for cross-infectivity. However, the isolates behaved differently; for example, *C. fioriniae* (KMI-2-2-Ca2), *C. fructicola* (ZYH-3-1-2-Ca1), and *C. siamense* (FuchuS-3-Cg2) infected all the tested plants, while *C. karsti* (YMZ-2-1-Cg1) and *C. aenigma* (STO-2-2-2-Cg2) failed to infect satsuma mandarin and peach. According to previous reports, all the species identified in the present study have a wide host range and worldwide distribution (Damm 2012a, 2012b; Jayawardena et al. 2021; Weir et al. 2012).

Colletotrichum fioriniae has been recorded to be associated with numerous plants including kiwifruit, persimmon, peach, and satsuma mandarin (Jayawardena et al. 2021; Lee et al. 2018, 2020; Poti et al. 2023; Sato et al. 2013; Tan et al. 2022; Tashiro et al. 2018; Xu et al. 2022). *Colletotrichum fructicola*, *C. siamense*, and *C. nymphaeae* have also been recorded in all the tested plants in this study, excepted satsuma mandarin (Carraro et al. 2019; Chang et al. 2017; Damm et al. 2020; Evallo et al. 2022; Hassan et al. 2019; Huang et al. 2022; Kim et al. 2018; Kuang et al. 2021; James et al. 2014; Lee et al. 2020; Moreira et al. 2020; Poti et al. 2023; Tan et al. 2022). *Colletotrichum aenigma* has been identified in more than 20 plant species including kiwifruit and persimmon (Andrioli et al. 2021; Far and Rossman 2023; Wang et al. 2019), but it is not found in peach, loquat, and satsuma mandarin, which is consistent with the pathogenicity tests of the present study. *Colletotrichum gloeosporioides* has been recorded in all the plants tested in this study (Deng et al. 2017; Huang et al. 2013; Li et al. 2017; Naz et al. 2017; Poti et al. 2023; Zhang 2008). However, the isolate from this study failed to cause symptoms on satsuma mandarin and peach. *Colletotrichum karsti* is known to be a pathogenic pathogen in numerous plants as well as peach, kiwifruit, and persimmon (Far and Rossman 2023; Poti et al. 2023; Tan et al. 2022; Wang et al. 2015); however, there is no report in loquat and satsuma mandarin. Overall, due to cross-infectivity of all the *Colletotrichum* species identified in loquat to other economic plants, disease management is necessary to prevent damage to loquat production as well as other economic fruits.

In conclusion, the seven species were identified in loquat in Japan and have different cross-infection properties across other economic plants. The findings gained from this study are crucial for epidemiology and provide a cautionary tale for disease management.

Declarations

Acknowledgement

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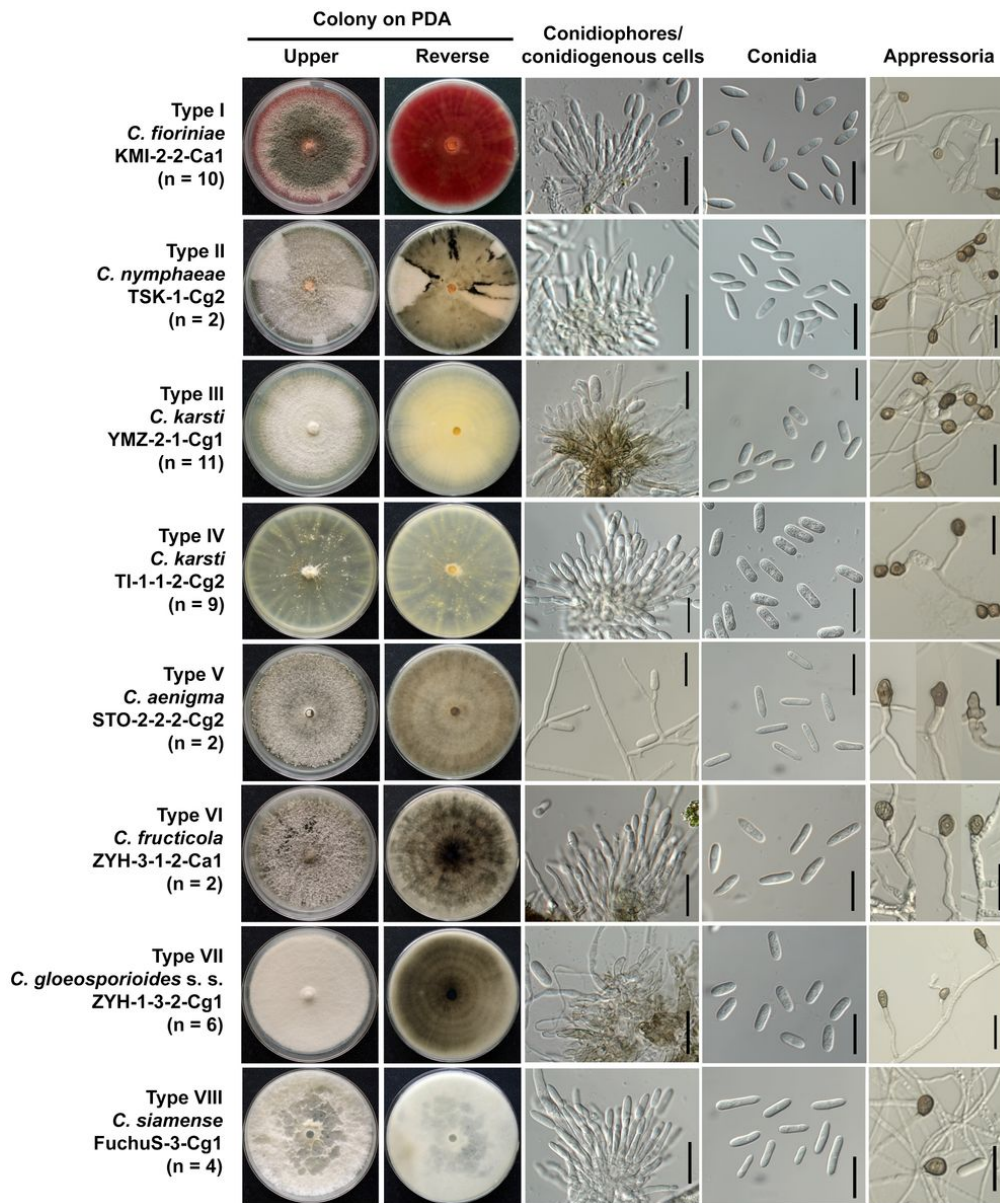
Figures

Figure 1

Culture and morphological characteristics of the *Colletotrichum* species isolated from loquat. Colony features on PDA at 25°C in the dark for 10 days. Scale bar is 20 μm.
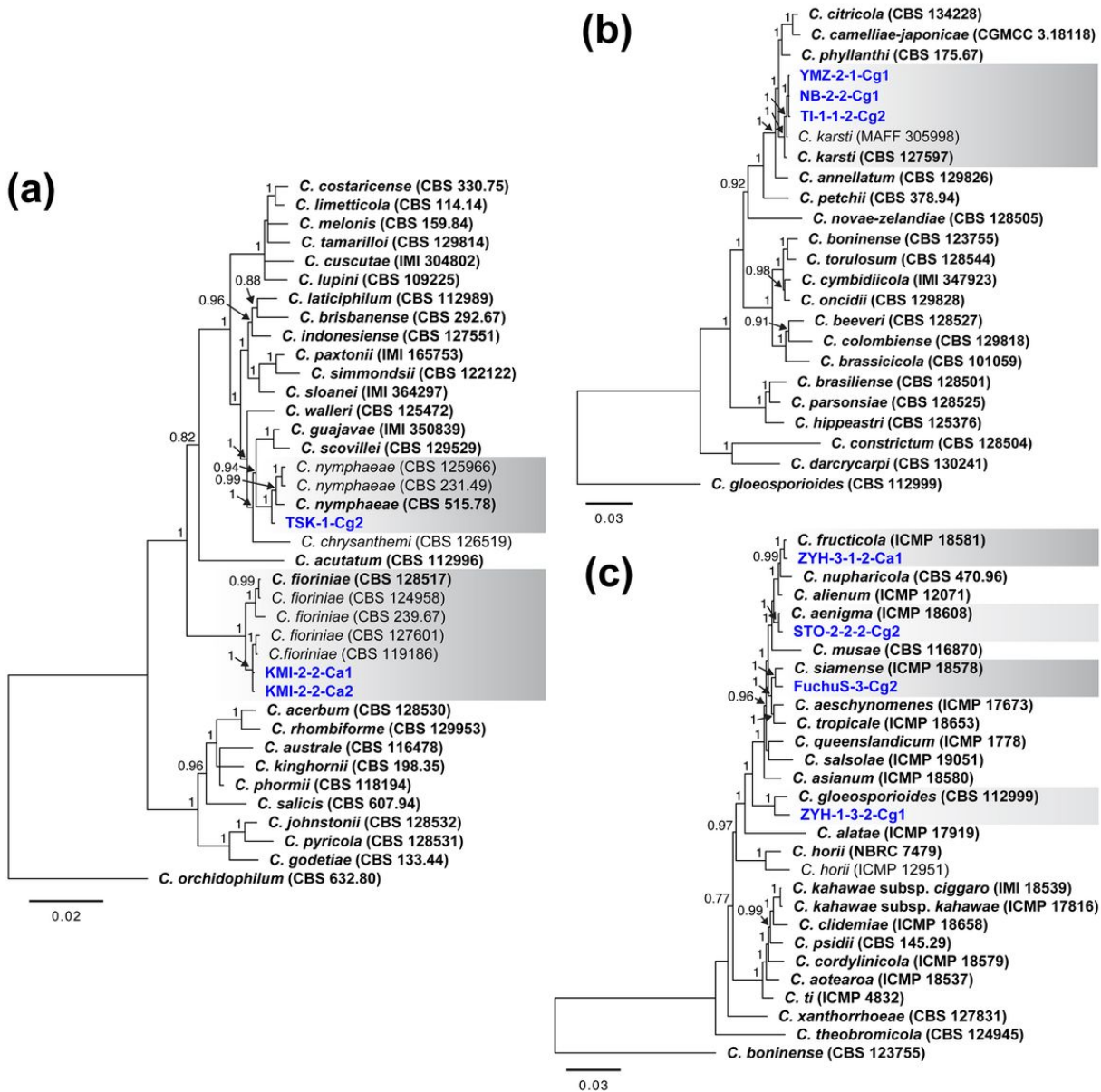
Figure 2

Bayesian inference phylogenetic trees of the *Colletotrichum* isolates associated with loquat anthracnose in Kagawa and Tokushima prefectures, Japan, with the species belonging to (a) the *Colletotrichum acutatum* species complex (CASC), (b) the *C. boninense* species complex (CBSC), and (c) the *C. gloeosporioides* species complex (CGSC). The trees were built based on concatenated sequences of ITS, *GAPDH*, *CHS-1*, *ACT*, and *TUB2* for CASC; ITS, *GAPDH*, *CHS-1*, *ACT*, *TUB2*, and *CAL* for CBSC; and *ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS for CGSC. Outgroups for CASC, CBSC, and CGSC were *C. orchidophilum* (CBS 632.80), *C. gloeosporioides* s. s. (CBS 112999), and *C. boninense* (CBS 123755), respectively. Ex-type species are indicated in bold and the isolates from this study are indicated in blue. Bayesian posterior probabilities above 0.7 are displayed at the nodes. The scale bar indicates the number of expected changes per site.
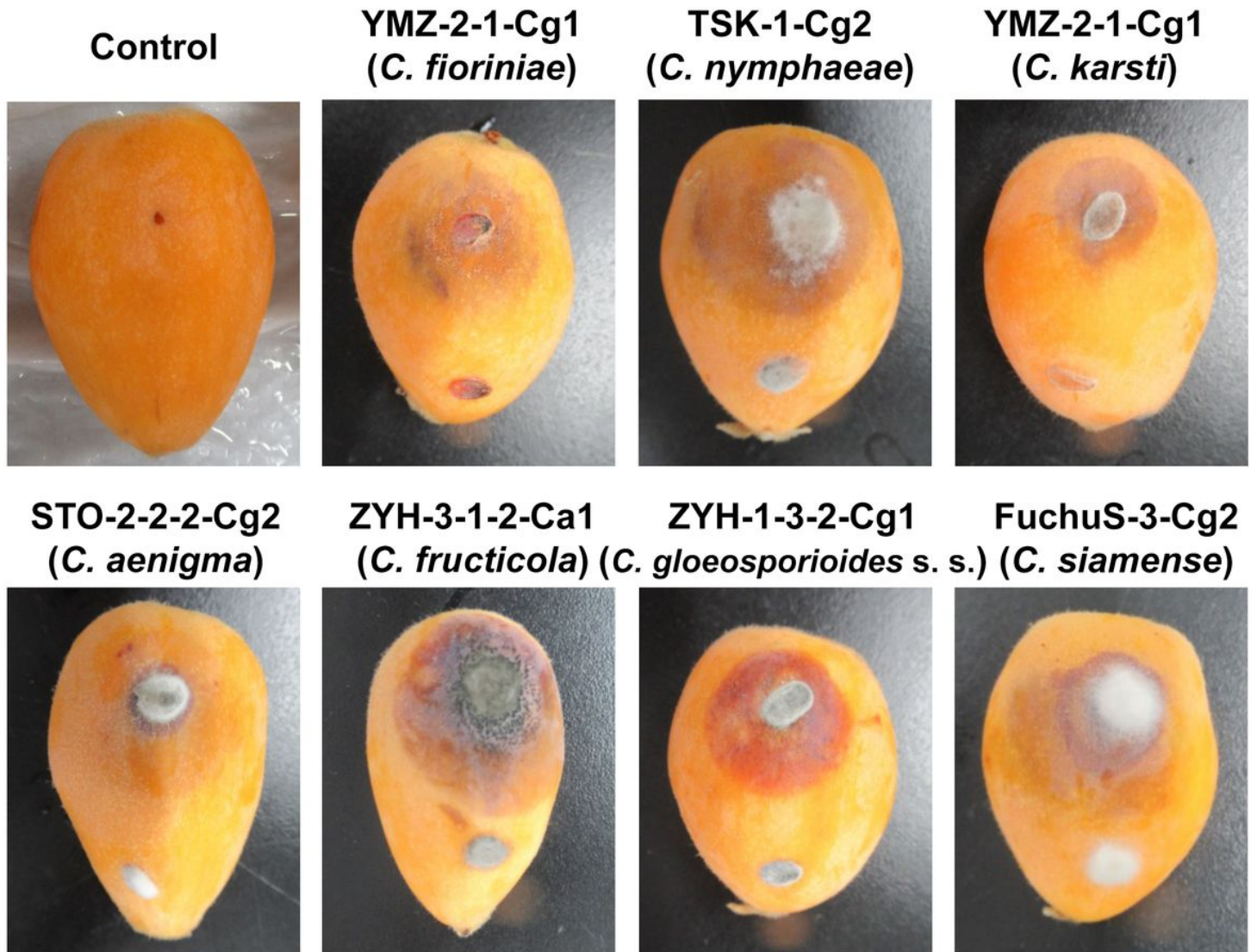
Figure 3

Results of inoculations with the *Colletotrichum* isolates YMZ-2-1-Cg1 of *Colletotrichum fioriniae*, TSK-1-Cg2 of *C. nymphaeae*, YMZ-2-1-Cg1 of *C. karsti*, STO-2-2-2-Cg2 of *C. aenigma*, ZYH-3-1-2-Ca1 of *C. fructicola*, ZYH-1-3-2-Cg1 of *C. gloeosporioides* s. s., and FuchuS-3-Cg2 of *C. siamense* on loquat fruits after inoculation for 7 days. The upper and lower sites are wounded and intact, respectively.
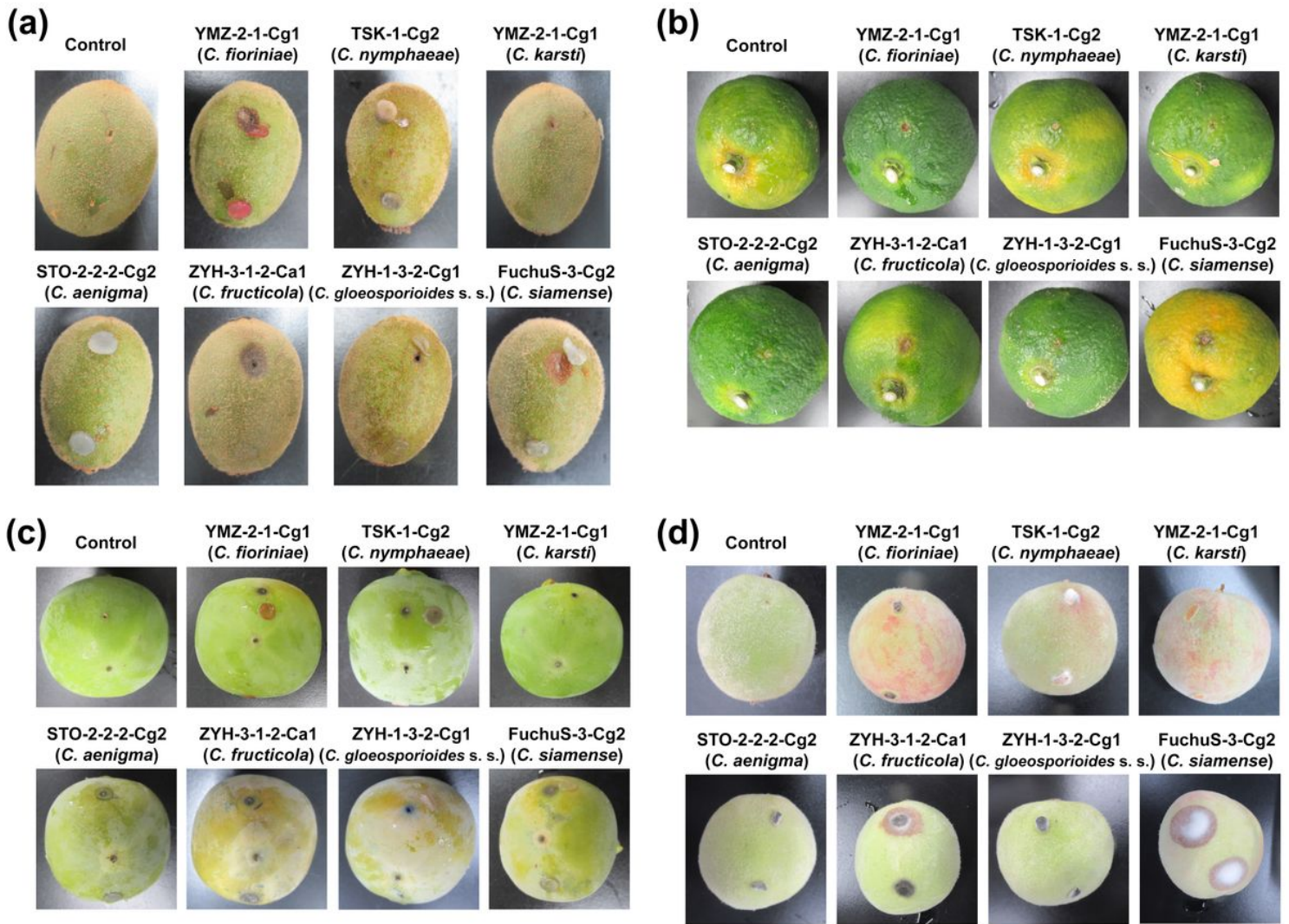
Figure 4

Results of inoculations with *Colletotrichum* isolates YMZ-2-1-Cg1 of *Colletotrichum fioriniae*, TSK-1-Cg2 of *C. nymphaeae*, YMZ-2-1-Cg1 of *C. karsti*, STO-2-2-2-Cg2 of *C. aenigma*, ZYH-3-1-2-Ca1 of *C. fructicola*, ZYH-1-3-2-Cg1 of *C. gloeosporioides* s. s., and FuchuS-3-Cg2 of *C. siamense* on kiwifruit (a), satsuma mandarin (b), persimmon (c), and peach (d) fruits after inoculation for 7 days. The upper and lower sites are wounded and intact, respectively.

Supplementary Files

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