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The variations of five Colletotrichum strains from strawberry in mycelial growth and pathogenicity below 26℃

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Research Article

Keywords: Colletotrichum spp., temperature, mycelial growth, pathogenicity, strawberry

Posted Date: March 7th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3982707/v1

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Abstract

Anthracnose caused by *Colletotrichum* spp. is a serious threat to many crops. *C. siamense* has been increasingly prevalent recently. However, the biology and pathogenicity of these fungi was largely elusive, especially below 26°C. Currently, the mycelial growth of five representative *Colletotrichum* strains including three *C. siamense*, one *C. fructicola* and one *C. gloeosporioides* s.s. strain from diseased strawberry was characterized at 10, 14, 18, 22 and 26℃. Their pathogenicity to two hosts under 14℃ and 22℃ was investigated. These strains did not differ much in mycelium growth between 10–26℃, although all *C. siamense* and *C. gloeosporioides* s.s. strains showed some superiority than *C. fructicola*. However, great variations were observed in their pathogenicity to *Fragaria vesca* and *F. × ananassa* cv. 'Benihoppe'. The strain Cs:GQHZJ19 with fastest mycelial growth at 22℃ was also the most virulent to both hosts under 14 / 22℃. But the mycelial growth rate was only positively correlated with *C. siamense* pathogenicity to the susceptible cv. 'Benihoppe' at 3 dpi under 22℃. Based on the pathogenicity analysis for five *Colletotrichum* strains on hosts with varying susceptibility under distinct temperatures, this work suggested that host genotype might play a decisive role in disease severity from the beginning of infection, while temperature could shape host-*Colletotrichum* spp. interaction at a later infection stage. Future studies with more fungal samples are required to determine the biology and virulence of *Colletotrichum* strains (especially *C. siamense*) in a wider temperature range, which will eventually facilitate an effective management of crop anthracnose.

Introduction

Strawberry is a nutritious and popular small fruit crop all over the world. It belongs to the genus *Fragaria* in the family *Rosaceae*, and there are four basic reproductive groups in the genus *Fragaria*, mainly varying with ploidy level or chromosome number (Hancock 1999). *Fragaria vesca* L., a diploid (2n = 2x = 14) strawberry, is a relative of the commercial octoploid strawberry, also the predominant sub-genome provider for octoploid strawberry (Oosumi et al. 2006; Edger et al. 2019). The octoploid strawberry *F.× ananassa* Duchesne (2n = 8x = 56) is the major cultivated group (Davis et al. 2007). *Rosaceae* is reported to be the family with the highest number of records associated with the filamentous fungal *Colletotrichum* genus, with as many as 41 *Colletotrichum* species being pathogenic to rosaceous plants (Talhinhas & Baroncelli 2021). Indeed, strawberry is susceptible to a wide range of phytopathogenic organisms and is considered as a model system for *Rosaceae* family (Amil-Ruiz et al. 2011).

As one of the oldest fungi on Earth co-evolved with flowering plants, the genus *Colletotrichum* consists of more than 280 species of *Ascomycota*, consisting of 16 species complexes and 15 additional singletons (Liu et al. 2022). *Colletotrichum* is a very dominant plant pathogen that causes anthracnose leaf blight or fruit/stem rot on more than 3,000 species of plants (O'Connell et al. 2012). Anthracnose, caused by *Colletotrichum spp.*, is a serious threat to strawberry industry, as anthracnose can infect a wide range of strawberry tissues, causing black spots on leaves, petioles, stolons, and fruits that are sunken or necrotic, leading to wilting of the entire plant due to crown rot (Howard et al. 1992). C. *siamense* and C. *fructicola* of C. *gloeosporioides* sensu lato are currently the most prevailing species infecting strawberries in Asia (Ji et al. 2022), which was closely related with their broad host range, vital environmental adaptation and powerful genetic flexibility (da Silva et al. 2020; Wilson et al. 2019).

Temperature is one of the key environmental factors affecting pathogen survival and aggressiveness (Velásquez et al. 2018). Epidemics caused by *Colletotrichum* spp. generally occur in a wet and warm weather, at temperatures between 20 ℃ and 30 ℃ (Ngugi et al. 2000; Sharma and Kulshrestha 2015; Soares-Colletti and Lourenço 2014). The temperature requirements of *Colletotrichum* fungi vary with the biological process, fungal genotype and ecotype (Chung et al. 2020; Moreira et al. 2021; Soares-Colletti and Lourenço 2014). The optimal temperatures for mycelial growth of *Colletotrichum* spp. largely range from 24℃ to 28 ℃ (Salotti et al. 2022). The favorable temperature for mycelial growth was proposed between 25.83℃ to 28.22℃ for several representative *Colletotrichum* strains from eastern China (Ji et al. 2023). *C. siamense* has been one of the most predominant species threatening strawberry worldwide with an advantageous tolerance to higher temperature especially in mycelial growth (Ji et al. 2022, 2023). Previous studies have described the morphological characteristics and the pathogenicity of different *Colletotrichum* species under high temperatures (Damm et al. 2014; Dubrulle et al. 2020; He et al. 2019; Ji et al.

2023; Munir et al. 2016; Weir et al. 2012). However, the biology and pathogenicity of *Colletotrichum* species especially the prevailing *C. siamense* under low-temperature conditions has not been studied yet. To bridge the gaps in the temperature requirements of the prevailing *Colletotrichum* species, thermal phenotyping was necessary under a low-temperature range. Such investigation would contribute to the risk assessment and management of *Colletotrichum* spp. in a wide range of temperatures.

The long-term goal of our lab is to provide useful information for the management of anthracnose. Our previous work (Ji et al. 2023) stimulated several questions. Does the strains of *C. siamense* with growth superiority above 30℃ have a relatively lower risk for crop below 26℃? Does the growth rate of *Colletotrichum* spp. correlate with their pathogenicity to strawberry under a lower temperature range? Again, we questioned the potential tradeoff between the tolerance to high temperature among *C. siamense* strains and their virulence to susceptible hosts as previously suggested (Chen et al. 2017; Ji et al. 2023). The specific objective of this study using five representative *Colletotrichum* strains was: i) to evaluate the potential risk of *Colletotrichum* spp. below 26℃ via modeling mycelial growth and pathogenicity analysis, ii) to examine the possible tradeoff between temperature adaption and fungal pathogenicity and iii) to compare the relative influence of temperature and host genotype on strawberry disease severity upon *Colletotrichum* infection.

Materials and methods

Plant materials and growth conditions

The woodland strawberry 'Ruegen' (*F. vesca*) and commercial octoploid strawberry 'Benihoppe' (*F. × ananassa* Duch) were cultivated in a strawberry greenhouse at Institute of Forestry and Fruit Tree Research, Shanghai Academy of Agricultural Sciences in current work. Stolon-derived 'Benihoppe' and seed-germinated 'Ruegen' seedlings were grown under 24 ± 2 ℃ with a 14-h-light/10-h-dark rhythm. Plants were regularly watered twice a week. Fertilizer (0.1%) containing N-P-K: 15-15-15 (YaraMila, Norway) was used every one week. Old leaves were removed followed by spraying fungicides. Fungicides and fertilizer were no longer applied from two weeks prior to inoculation. The third and fourth leaves were freshly detached and used for site-specific fungal inoculation or mock treatment.

Fungal strains

Three strains of *C. siamense* GQHAH2, Nj-2 and GQHZJ19 with varied virulence isolated from strawberry gardens in Anhui, Jiangsu and Zhejiang Provinces of eastern China (Han et al. 2016; Zhang et al. 2020), respectively, were selected for current research. An additional strain belonged to *C. gloeosporioides* sensu stricto (s.s). JSH-7-1 isolated from diseased strawberry in Hubei (Han et al. 2016). One strain of *C. fructicola* with strong virulence maintained in current lab, was isolated from diseased strawberry in Shanghai (Ren et al. 2008) and later deposited under accession no. as CGMCC3.17371 (Zhang et al. 2016).

All five strains of three species belong to the *C. gloeosporioides* complex. These fungi were rejuvenated in 2022 via isolating single spore colonies from the infected leaf tissues of strawberry cv. '*Benihoppe*'. Before testing under varying temperatures, each strain was propagated on PDA plates for 7 days at 28°C in darkness.

Characterizing the growth of mycelium

The 7-day-old culture of each strain was scribed at the advancing edges with a sterilized scalpel and three mycelial pieces in a size of 1–2 mm^2 were transferred to a new plate (Φ = 90 mm) with fresh PDA media. For each temperature × strain combination, four replicate plates were set, each with three 1–2 mm^2 fresh marginal mycelia. A total of 20 plates per temperature were placed according to a completely randomized block design in a low-temperature incubator (KT053, Binder, Germany). The culture conditions were in the darkness under 10, 14, 18, 22 or 26°C.

After 3 d incubation under each temperature, both sides of the colonies were photographed. Simultaneously, the morphological characteristics including the texture, density, color, and the shape of the mycelial colonies were visually recorded. Furthermore, colony diameter was measured using ImageJ software (Rueden et al. 2017). A total of 12 data per strain per temperature were

obtained for modeling and statistical analysis. The rate of mycelial extension was calculated as the average growth in 3d culture (mm/day). The experiment was independently repeated in 2022 and 2023.

Preparation of inoculum

About 10 mycelial squares in sizes of about 1cm×1cm were cut from the advancing edges of the fresh 7-d-old plates incubated under 14 or 22℃. These squares were transferred to a 250 ml conical flask with 100 ml PDB media. Conidia were produced in a shaker at 200 rpm for 4–7 days under 14℃ or 22℃, the same temperature as for mycelial culture. Then, the liquid culture collection was filtered through two-layered lens wiping tissues, quantified with a hemocytometer under Nikon Eclipse E200 microscope. Later, the solution was adjusted to 2×10^6 conidia per ml either by dilution with distilled water or enrichment via centrifugation. Under 22℃, the conidia inoculum of all strains except for Cs: Nj2 was adjusted via dilution. By contrast, the conidia inoculum was prepared via centrifugation enrichment under 14℃ for all *Colletotrichum* strains.

Pathogenicity assay

The pathogenicity of *Colletotrichum* strains has been studied by inoculating wounded leaves, largely following the method as previously reported (Ji et al. 2023). The six treatments consisted of five fungal strains and one mock treatment (sterile water containing 0.01% (v/v) Tween-20). Each strain was inoculated on two sites of the left side on 15 leaflets as well as the low right of 12 additional leaflets per temperature × strawberry variety combination, including the third and fourth leaflets of at least eight plants. Each leaflet was inoculated with two 10μl drops (2×10^6 per ml) of one conidial suspension on the left side, while 10 μl of mock solution and 10 μl of mock or an additional conidial suspension were inoculated on the upper right and low right, respectively. A total of 42 inoculation sites were studied per strain × temperature × strawberry variety.

After inoculation, petri dishes with leaflets were incubated in a dark incubator at 14 or 22°C (the same temperature for mycelial culture and conidia production) for 48h, followed by a 14h/10h light/dark cycle. Plant materials were photographed and recorded at 3 days or 5 days post inoculation (dpi). After the lesion size was calculated using ImageJ software, disease grading and disease index (%) were determined following rules described previously (Ji et al. 2014; Zhang et al. 2020). Experiment was carried out twice in 2023 independently.

Model fitting and statistical analysis

To reveal the distinct responses of *Colletotrichum* strains to temperatures (10–26℃) in mycelium growth, two-way ANOVA (Tukey-test, $P \leq 0.001$) was performed. Mycelium growth rate was compared between fungal strains by Duncan's two factor test ($P \leq 0.05$). A polynomial model was used to establish a response curve for the growth rate of *Colletotrichum* mycelium at different temperatures. The mycelial growth rate of each strain at different temperatures was analyzed using linear regression analysis in Statistical Product and Service Solutions (SPSS), modelled with a quadratic function, and written as:

$$Y(T) = b0 + b1*T\text{^}1 + b2*T\text{^}2 \quad (1)$$

where T is the independent variable temperature, Y is the dependent variable mycelium growth rate, and three parameters of the model include $b0$ the intercept, $b1$ and $b2$ the coefficients of T and T-squared.

In addition, a linear regression analysis was performed to evaluate the relative influence of 'temperature' and 'host genotype' on the disease severity caused by *Colletotrichum* pathogen in strawberry, which was modelled with a linear function and written as:

$$Y = a*X + b \quad (2)$$

where Y is disease index as the dependent variable, X is temperature or host genotype as independent variables, a is the coefficients for certain independent variable (slope) and b is the constant of the equation (intercept).

Parametric correlation evaluation between 'mycelium growth rate' (under 14/22℃) and *Colletotrichum* pathogenicity ('disease index') (in *F. vesca* / *F. × ananassa*), and nonparametric correlation for 'temperature', 'host genotype' and 'fungal ecotype' with

'disease severity' were performed using the CORREL analysis in SPSS. The SPSS 25.0 statistics was used for the regression and statistical analysis.

Results

Mycelium morphology of strawberry-pathogenic Colletotrichum strains under 10°C – 26°C

Five representative *Colletotrichum* strains from eastern China were selected for mycelium characterization after 3d-incubation in darkness on PDA at 10 to 26°C (Fig. 1). Differences in the colony morphology of these five strains could be observed under all temperatures tested except for 10°C, although the mycelial growth trend of the five fungi did not differ much. After three days' incubation under 14°C, Cs:GQHZJ19 developed relatively denser mycelia while Cf: CGMCC3.17371 formed thinner mycelia than the rest fungal strains.

Under 18–26°C, *C. siamense* and *C. gloeosporioides* s.s. were largely greyish-white with abundant mycelium, intact margin, and a pale orange dorsal surface. At 18°C, the plates of all five strains were greyish-white with markedly elevated centers, although their reverse sides uniformly displayed an orange center except for Cf: CGMCC3.17371. Under 22°C, *C. siamense* GQHZJ19 produced white dense mycelium with a gradually declining outside edge together with brown conidial mounds forming in the center, which was much clear than that at 18°C and 26°C. Generally, Cs:Nj2 looked like Cf: CGMCC3.17371 under 22–26°C, although a relatively faster growth was observed in the former. In fact, the colonies of *C. fructicola* at 18–26 ℃ were greyish white, actively growing, with relatively sparse and fluffy edges, and the mycelium was visible on the back of all plates in a clockwise spinning growth. There was no discernible difference between the colonies under the conditions of 22℃ and 26℃.

Morphologically, *C. gloeosporioides* s.s. JSH-7-1 was basically the same as *C. siamense* GQHAH2 under 18–26℃, but the mycelium of Cg:JSH-7-1 was more abundant and compact than Cs:GQHAH2.

The above observations were largely coincident with a previous work (Ji et al. 2023), although clear differences were observed for Cs: GQHZJ19 and Cf: CGMCC3.17371. In that work, Cs:GQHZJ19 colony was markedly raised in the center under 22℃ and 26℃, which was not observed in current work. For *C. fructicola*, olivaceous gray flocculent in the center was observed in our previous work but not in current work. Fungal morphology is easily affected by cultivation, and these minor differences might be interpreted from distinct incubation duration, different starting materials or unknown factors.

Mycelial growth rates of Colletotrichum strains under different temperatures

The average mycelial growth rates of each strain were quantified from 10 to 26℃ (Fig. 2). Significant differences among *Colletotrichum* strains were detected under all temperatures tested. At 10℃, the mycelial growth rate of Cf: CGMCC3.17371 was slower than that of Cs:Nj2 (Fig. 2a). Indeed, the mycelia of Cf: CGMCC3.17371 grew at the lowest rate under all temperatures in this study. Generally, the rest strains grew at similar rates without significant differences. However, the mycelial growth rates of Cs:GQHAH2, Cg:JSH-7-1 and Cs:GQHZJ19 peaked at 14℃, 18℃ and 22℃, respectively. Morphologically similar, Cg:JSH-7-1 and Cs:GQHAH2 grew at similar speed under 22–26℃, while their growth rates were significantly different from each other under 14 and 18℃. Another pair Cf: CGMCC3.17371 and Cs:Nj2 grew at significantly distinct rates under all temperatures except for 22℃ (11.89 and 12.38 mm/d). The growth rate of Cs:GQHZJ19 ranked under 22°C (13.30 mm/d) and 26°C (14.13 mm/d), but a significant difference could be observed only under 22°C.

Following the increase of temperature below 22°C, a sharp increase in the rate of *Colletotrichum* mycelial growth was uniformly observed in all five strains (Fig. 2b). With the exception of Cf: CGMCC3.17371, both *C. siamense* and *C. gloeosporioides* strains grew at a higher speed from 22°C to 26°C, although the increase was minor than that observed below 22°C. Statistics analysis suggested that all four *C. siamense* and *C. gloeosporioides* strains have similar thermal responses during 10–26°C, while the growth response of Cf: CGMCC3.17371 to temperature changes was significantly different from the rest strains.

These observations were not completely meeting with our previous study (Ji et al. 2023). In that work, the average mycelial growth of these five strains were not significantly different from each other during five days under 22°C. While incubated at 26°C for four days, Cf: CGMCC3.17371 and Cg:JSH-7-1 grew at a speed significantly higher than that of Cs:GQHAH2. In a way, current work provided more accurate comparison and distinguishment for the hypha growth of these *Colletotrichum* strains in an identical duration (3 d).

Polynomial curve fitting for the temperature responses of Colletotrichum mycelium growth

The generalized *beta*-function of nonlinear regression model (Hau et al. 1985) has been successfully used for fitting *Colletotrichum* mycelium growth in relation to temperatures from 22°C to 36°C (Ji et al. 2023). Currently, a series of derivations in SPSS software indicated that the generalized *beta*-function was not applicable to the low temperature interval from 10°C to 26°C (data omitted). As the growth of fungi plotted against temperature might follow a quadratic parabola (Hau and Kranz 1990), a polynomial function was used to fit the mycelial growth rates in this study (Eq. 1, Fig. S1, Table 1). The coefficient of determination (R^2) value was an index of model fitting used to quantify the strength of the relationship between the response variable and the dependent variable. As shown in Table 1, all R^2 and adjusted R^2 values in current modelling were greater than 0.95.

Table 1
Response surfaces for mycelium growth rate of different *Colletotrichum* strains as a function of temperature described by the equation: Y = b0 + b1*X^1 + b2*X^2

Fungal strains	*Cs*:GQHAH2	*Cs*:Nj-2	*Cs*:GQH ZJ19	*Cf*:CGMCC3.17371	*Cg*:JSH-7-1
b0	-14.48051 ± 0.99366	-12.42774 ± 1.20755	-12.94563 ± 1.2914	-13.30115 ± 1.49075	-16.71172 ± 0.98774
b1	1.80004 ± 0.11816	1.51054 ± 0.14359	1.52446 ± 0.15356	1.61068 ± 0.17859	2.07545 ± 0.11745
b2	-0.02732 ± 0.00325	-0.019 ± 0.00395	-0.01773 ± 0.00423	-0.02393 ± 0.00495	-0.03498 ± 0.00323
Sum of squared residuals	25.95995	38.3392	43.84781	51.98874	25.65142
R^2	0.98059	0.97192	0.97199	0.95251	0.9811
Adjusted R^2	0.97991	0.97094	0.97101	0.95069	0.98044

In this experiment, the fastest mycelial growth was observed at 26°C, when the model curve still showed an increasing trend for all *Colletotrichum* strains (Fig. S1), which was consistent with their optimum temperature above 26°C proposed based on the generalized *beta*-function (Ji et al. 2023). The growth trend of *C. fructicola* strain was obviously the lowest at different temperatures. *C. gloeosporioides* s.s. growth displayed minor advantage during 10–22°C, which was not easily distinguished from the patterns of *C. siamense*. From 26°C on, three *C. siamense* strains consistently exhibited slightly faster trend in radial mycelium growth than *C. gloeosporioides* s.s.. In brief, the polynomial fitting conformed to the quadratic function curve shed lights on the relative growth superiority of these *Colletotrichum* strains and the corresponding temperature ranges.

Pathogenicity of Colletotrichum strains to two strawberry hosts under 14°C and 22°C

The pathogenicity of five *Colletotrichum* strains were investigated on two strawberry hosts with varying susceptibility under 14°C and 22°C. All strains were pathogenic on the wounded leaves of *F. vesca* and *F. × ananassa*, but the resulted disease severity varied with temperature, host type, fungal strain and time post inoculation. No necrotic lesions occurred on mock-treated leaves of two strawberries at any phase after inoculation, and leaves with typical symptoms were displayed in Fig. 3.

Under 14°C, black necrosis was hardly observed on *F. vesca* at 3 dpi, and minor necrotic points could be discernible only at 5 dpi after inoculation with Cs:GQHZJ19 and Cf:CGMCC3.17371. By contrast, under the same temperature, on the leaves of the

highly susceptible cultivar ‘Benihoppe’, necrosis was obviously present at 3 days post inoculation with Cs:GQHZJ19. Indeed, needle-like necrotic points were also discernible on ‘Benihoppe’ at this stage post inoculation with the rest two *C. siamense* strains. Till 5 dpi, all five *Colletotrichum* strains caused necrosis on ‘Benihoppe’ leaves at 14°C, although three *C. siamense* triggered significantly larger lesions than *C. fructicola* and *C. gloeosporioides* s.s.

Under 22°C, inoculation with Cs:Nj2, Cs:GQHZJ19 and Cf:CGMCC3.17371 resulted in needle-like necrotic points on the leaves of *F. vesca* at 3 dpi. Till 5 dpi, necrosis lesions were easily observed on *F. vesca* inoculated with all strains except for Cs:GQHAH2. Comparatively, the disease symptoms on *F. vesca* at 5 dpi were somewhat equivalent to that observed at 3 dpi on ‘Benihoppe’. Actually, the virulence of each fungal strain was remarkably pronounced on the wounded leaves of ‘Benihoppe’ at 5 dpi under 22°C. Both *C. fructicola* and Cs:GQHZJ19 triggered necrotic symptoms at all 42 inoculation sites per strain after three days’ inoculation at 22°C on ‘Benihoppe’. Up to 5 dpi at 22°C, the number of necrotic spots increased, the area of the necrosis expanded, and every fungus caused necrotic lesions on all 42 inoculation sites per strain.

The above observations were preliminary based on several typical infected leaves. In current work, two independent replications provided generally consistent results on the relative pathogenicity of these fungal strains at certain time post inoculation under distinct temperature and for distinct hosts. Together with disease index calculated from necrosis rating values of all 42 inoculation sites, the percents of disease degree were summarized to show the overall pathogenicity per temperature× host type× fungal strain at each phase of one independent experiment (Fig. 4). Clearly, low temperature such as 14°C limited the pathogenesis in two hosts, while 22°C temperature optimum for both host and pathogen fostered the development of anthracnose necrosis. The pathogenicity of these strains varied with host type, infection duration and temperature.

At 3 dpi under 14°C, the virulence of five strains was too weak to be distinguished from each other on the less susceptible *F. vesca*. For the same host plant at 3dpi, the relative virulence of five strains under 22°C could be ranked as follows: Cs:GQHZJ19 > Cf:CGMCC3.17371 > Cs:Nj2 > Cg:JSH-7-1 > Cs: GQHAH2. Till 5 dpi on the less susceptible host, the relative pathogenicity of five strains largely ranked as Cs:GQHZJ19 > Cf:CGMCC3.17371 > Cs:Nj2 & Cs:GQHAH2 > Cg:JSH-7-1 independent of temperature. It was hard to tell the virulent one from *C. siamense* strains Cs:NJ2 and Cs:GQHAH2 for *F. vesca*.

For the highly susceptible commercial cultivar ‘Benihoppe’, Cs:GQHZJ19 still was the most virulent strain and manifested the highest pathogenicity (Fig. 4). At 3 dpi under either 14°C or 22°C, CGMCC3.17371, Cs:Nj2 and Cs:GQHAH2 triggered similar disease incidence on ‘Benihoppe’, a little more severe than that of Cg: JSH-7-1 and weaker than that of Cs:GQHZJ19. Till 5 dpi, the virulence of five strains except for Cs:GQHZJ19 to ‘Benihoppe’ was largely similar under 14°C. For ‘Benihoppe’, the relative virulence of five strains were the same as that for *F. vesca* under 22°C at 5 dpi.

Correlation of mycelial growth with C. siamense pathogenicity under 14°C and 22°C

The above work indicated that the mycelial growth rate of selected *C. fructicola* strain was always the lowest while its virulence was significantly higher than that of *C. gloeosporioides* s.s. strain. Clearly, mycelial growth rate did not correlate with *Colletotrichum* strains across different species. Since the delayed mycelial growth might contribute to the reduction in the pathogenicity of *C. gloeosporioides* (Zhang et al. 2023), the potential correlation of mycelial growth rate with fungal pathogenicity (disease index) was evaluated among strains of *C. siamense*. Parametric correlation analysis was performed in SPSS software (Table 2).

Table 2
Parametric correlation analysis of mycelial growth and the pathogenicity of *C. siamense*

Parameter	*F. vesca* var. *Ruegen*				*F.* × *ananassa* cv. *Benihoppe*			
	14°C		22°C		14°C		22°C	
3dpi	5dpi	3dpi	5dpi	3dpi	5dpi	3dpi	5dpi	
Correlation coefficient[#]	-0.984	-0.651	0.855	0.958	-0.695	-0.644	**0.997***	0.959
P	0.113	0.549	0.347	0.186	0.511	0.554	**0.045**	0.183

[#]Correlation coefficient for the mycelial growth rate and the disease index caused by *C. siamense.*

* The correlation was significant (P < 0.05) (2-tailed)

Interestingly, the mycelial growth rate of *C. siamense* was always negatively correlated at 14°C and positively correlated at 22°C with its pathogenicity to strawberry under the same temperature, independent of host susceptibility degree and time post inoculation, although not at a significant level in most cases. The only exception was that, the mycelial growth rate of these fungal strains at 22°C was significantly and positively correlated with the disease index on the highly susceptible cultivar 'Benihoppe' at three days post inoculation with *C. siamense*.

Influences of temperature, host genotype and fungal ecotype on Colletotrichum pathogenicity

Furtherly, correlation relationship among "temperature", "host genotype", "fungal ecotype" and "disease index" were investigated via using non-parametric correlation analysis in SPSS. Similar results were obtained when data of all five *Colletotrichum* strains or only three *C. siamense* strains were analyzed. Evaluation results for all five strains belong to three *Colletotrichum* species were shown in Table 3. When the disease index was treated as the dependent variable reflecting fungal pathogenicity, fungal ecotype was not correlated with the pathogenicity at early (3 dpi) or late (5 dpi) infection stage. Notably, "host genotype" was always significantly correlated with disease index, at either early or late infection stage. In contrast to this, 'temperature' only significantly correlated with fungal pathogenicity at 5 dpi the late infection stage. This analysis hinted that at the early infection stage, host genotype was the crucial factor holding an extremely significant effect on pathogenesis in a temperature range around 14°C ~ 22°C.

Table 3
Nonparametric correlation of 'temperature', 'host genotype' and 'fungal ecotype' with *Colletotrichum* pathogenicity ('disease severity')

			3dpi			5dpi		
Method	Dependent Variable	Parameter	Temperature	Host genotype	Fungal ecotype	Temperature	Host genotype	Fungal ecotype
Spielman Rho	Disease index	Correlation coefficient	0.305	**0.638****	0.000	**0.538***	**0.486***	0.018
		Sig. (2-tailed)	0.112	**0.001**	1.000	**0.014**	**0.030**	0.939
		N	20	20	20	20	20	20

*. At the 0.05 level (2-tailed), the correlation was significant.

**. At the 0.01 level (2-tailed), the correlation was extremely significant.

At 5 dpi, both host genotype and temperature were significantly correlated with disease index. To assess the influence of 'temperature' and 'host genotype' on disease severity statistically, a regression analysis was performed following a linear function (Eq. 2). From Table 4 it could be seen that the t-value of the analyzed 'temperature' was 4.670, the t-value of 'host

genotype' was 2.306 and the p-value for two factors were less than 0.05, hinting that both 'temperature' and 'host genotype' had a significant effect on 'disease index'. VIF value less than 5.0 indicated that the two regression lines were not covariant. *Beta* was the standardized coefficient for current regression modelling. The larger *Beta* value indicated that the dependent variable was more responsive to the independent variable, that is, the greater the degree of influence. Obviously, at late infection stage, 'temperature' has a greater influence (*Beta* = 0.703) on pathogenicity (disease index) than 'host genotype' (*Beta* = 0.347). The adjusted R^2 value suggested that 'temperature' and 'host genotype' could explain at least 56.9% variation in *Colletotrichum* fungal pathogenicity to strawberry at late infection stage.

Table 4
Influence of 'temperature' and 'host genotype' on 'disease index' at 5 dpi described as a function: Y = a*X + b

	Non-standardized coefficients[1]		Standardized coefficient[2]			
	b	**a**	**Beta**	**t**	**p**	**VIF**
Constant	-57.690 ± 16.087			-3.586	0002	
Temperature		3.415 ± 0.731	**0.703**	4.670	0.000	1.000
Host genotype		13.490 ± 5.850	**0.347**	2.306	0.034	1.000
R^2	**0.615**					
Adjusted R^2	**0.569**					

[1] *b*: the constant of the equation. *a*: the coefficients for certain independent variable. Dependent variable: disease index (Y).

[2] *Beta* is the standardized coefficient of regression modelling. R^2 value is the coefficient of determination, an indicator of model fit, the closer the value is to 1 the better the fit is.

Discussion

In this work, mycelial growth modelling based on a polynomial linear regression analysis revealed that *C. siamense* strains share similar growth trends with a *C. gloeosporioides* strain during 10–26°C, while significantly different from a *C. fructicola* strain. Indeed, our current and previous work (Ji et al. 2023) together suggested that *C. siamense* has prominent vigor during 10–40°C, and the thermotolerant strain Cs:GQHZJ19 also grew fast under 22/26°C.

In current work, Cs:GQHZJ19 was found to be highly pathogenic to both *F. vesca* and *F. × ananassa* at 14°C and 22°C. And the relative pathogenicity of three *C. siamense* strains was largely consistent with previous works on 'Benihoppe' at 25°C, i.e., GQHAH2, Nj2 and GQHZJ19, being weakly pathogenic, moderately pathogenic and strongly pathogenic, respectively (Ji et al. 2023; Zhang et al. 2020). However, Cs:GQHAH2 displayed significantly higher virulence to 'Benihoppe' than Cs:Nj2 at 5 dpi under 30°C (Ji et al. 2023). Since Cs:GQHZJ19 grew fastest in a wide range of temperature over 22°C, these observations rejected the previous arguments for a potential tradeoff between the fungal tolerance to high temperature and its virulence to susceptible host (Chen et al. 2017). Accordingly, it was proposed that *C. siamense* whose prevalence is increasingly favored in a global warming context, could do harm to host crops under a wide range of temperatures and will not reduce its potential threaten to agricultural production below 26°C.

Plants and pathogens share a co-evolution full of arms race pronounced as simultaneous development of host resistance and pathogen attack mode (Zhan et al. 2014, 2015). Throughout the co-evolutionary history, plants have evolved qualitative and quantitative resistance to improve their survival when confronted with pathogens (Poland et al. 2009; Lo Iacono et al. 2012). Host resistance is one of the most important biological factors shaping not only the populations but evolutionary structure of pathogens (McDonald and Linde 2002). Current statistic analysis enabled the evaluation of factors affecting *Colletotrichum* risk on strawberry. Our analysis supported that host genotype always significantly affected *Colletotrichum* pathogenicity. The decisive role of strawberry genotype in defeating *Colletotrichum* fungi was especially prominent at the early infection stage.

Again, breeding novel strawberry with improved resistance to *Colletotrichum* fungi is the best resolution for managing anthracnose in field.

Temperature is a crucial environmental factor affecting almost all aspects of biological and biochemical processes (Nadeem et al. 2014; Park et al. 2011). Concerning for the interaction between strawberry and *Colletotrichum* fungus, current work suggested that 'temperature' might not hold a significant influence at the early infection stage at 14/22°C. However, 'temperature' surpassed 'host genotype' (resistance) at the late infection stage in determining the final outcome of pathogen-host interaction. In addition, fungal ecotype was not correlated with anthracnose severity.

This preliminary work tried to address questions from previous work based on selected five representative *Colletotrichum* strains. To evaluate the biology and risk (virulence) of *Colletotrichum* strains (especially *C. siamense*) furtherly, more fungal strains are required for characterization in a wider temperature range. Moreover, future investigation on the temperature requirements of *C. siamense* in sporulation, conidial germination, and the formation of mycelial appressoria might provide a complete and clear scene on the thermal biology and effective management of this species.

Declarations

Acknowledgements

The authors gratefully acknowledge the assistance of Zi-Yi Li and Ying Ji for their discussion and guidance in fungal culture, diameter measuring, inoculation and modeling. We thank Ju-Fang Dai and Xian-Hua Dai for their supports in the establishment of plug seedlings. We would also like to show our gratitude to Cai-Ying Wu and Ji-Hua Jin for their assistance in the lab work. Thanks are due to anonymous reviewers for valuable comments which refined this manuscript.

Author Contributions: XL, CG, KD and QG conceived the study and designed the experiments. XL drafted the manuscript. XL, XH, SC, JY and FL performed experiments. XL, CG, YH and KD performed data analysis and interpretation. KD and QG contributed to revision of the manuscript. All authors approved the submitted version.

Funding

This work was partially funded by Science and Technology Commission of Shanghai Municipality (Key Program, 20392001500).

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval The manuscript does not contain experiments using animals and human studies.

Data Availability Statement: The datasets generated during and analyzed during the current study are available from the corresponding author Ke Duan (kduan936@126.com) on reasonable request.

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Figures

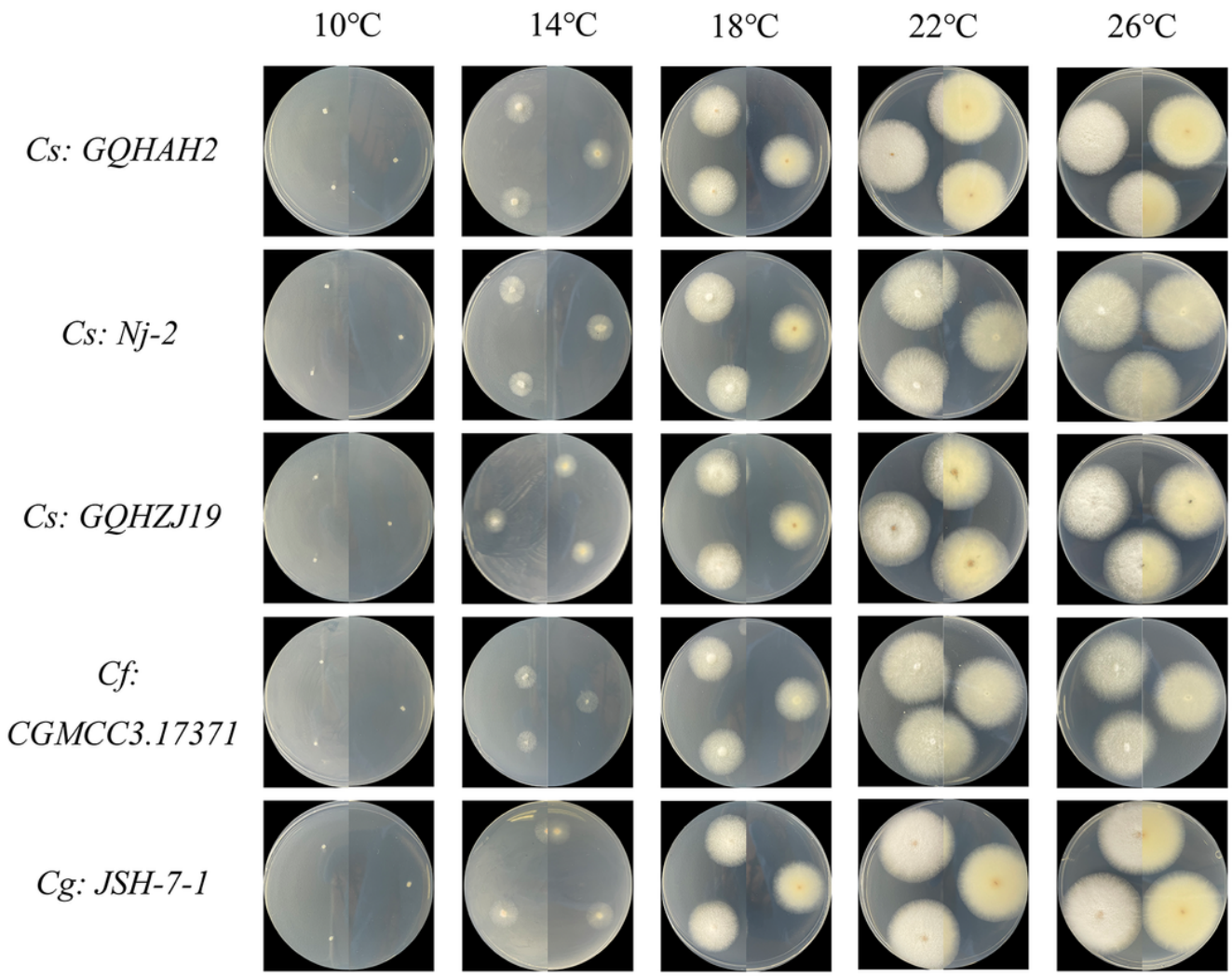


Figure 1

Typical colony morphology of strawberry-virulent *Colletotrichum* strains from eastern China. Five *Colletotrichum* strains including three *C. siamense* (GQHAH2, Nj-2, GQHZJ19), one *C. fructicola* (CGMCC3.17371) and one *C. gloeosporioides* sensu stricto (JSH-7-1) were studied. A sterile scalpel was used to scribe fresh hypha on the edges of *Colletotrichum* colonies incubated at 28°C for 7 days, and three fungal clumps of approximately 1-2 mm^2 in size were transferred to a new 90-mm-diameter PDA plate for test. These plates were incubated at 10°C, 14°C, 18°C, 22°C and 26°C in dark for exact three days. Each of these square plots consists of two patches stitched together, with the front of the plate on the left and the back of the plate on the right

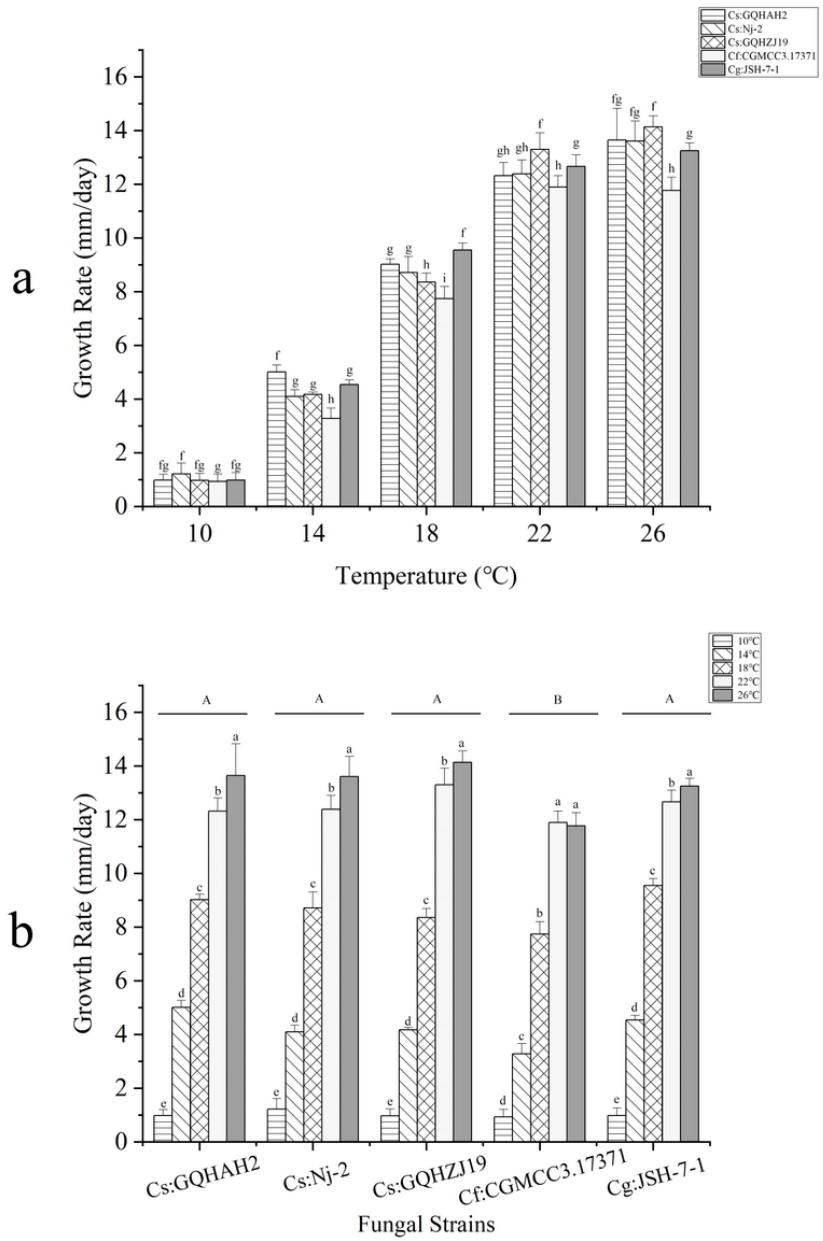


Figure 2

Colony growth rates of five *Colletotrichum* strains pathogenic to strawberry under 10-26 ℃. After the strains were incubated for three days at different temperatures in darkness, the diameter of each colony was measured. Data shown are means ± s.d. (n=12). **a** Difference among the growth rates of five fungal strains under the same temperature. According to Duncan's test, different lowercase letters indicates significant differences among strains at that temperature (p 0.05). **b** Distinct responses of *Colletotrichum*strains to varying temperature. Different lowercase letters indicate significant difference among growth rates under different temperatures for certain fungus (p<0.05). According to two-way ANOVA (Tukey-test), distinct uppercase letters marked over columns indicate significant differences among the thermal responses of five fungal strains (**P < 0.001)

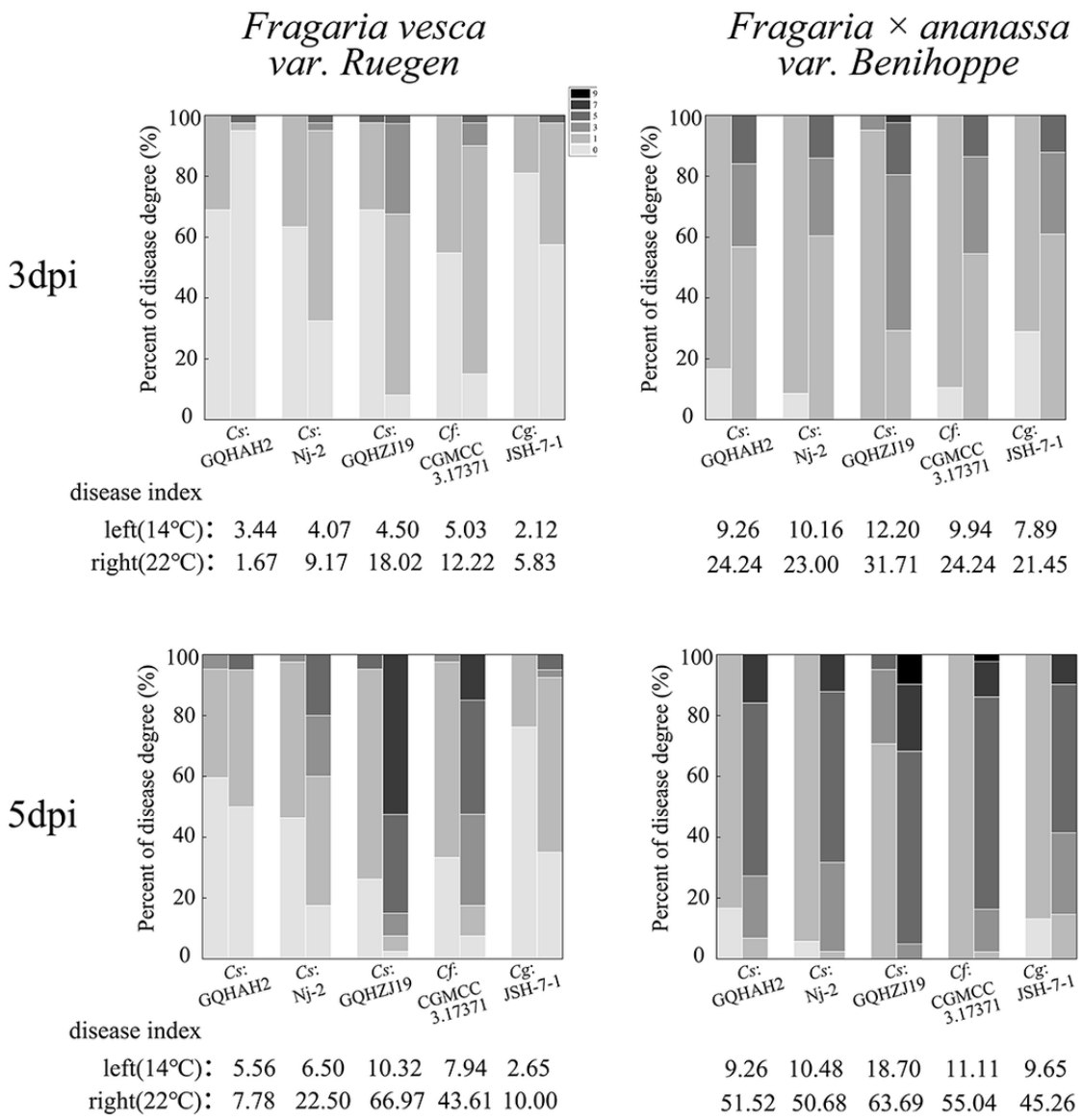


Figure 3

Disease symptoms on the wounded strawberry leaves inoculated with *Colletotrichum* spp. at different temperatures. Necrosis development after inoculation under 14°C or 22°C was photographed at 3 or 5 days post inoculation (dpi). Two sites on the left side of each leaf blade were inoculated with 10 µl (2×10^6 per ml) of conidial suspension of one strain. The mirror image of the site-specific inoculation with another fungal strain was cohered as the right half. Actually, there were two inoculation sites on the left and right sides of each leaf blade, where the left side was inoculated with two -drops of one conidial suspension, and the right side was inoculated with 10 µl of control sterile water (upper right) and 10 µl of an additional conidial suspension (low right) (three triplicates for certain additional strain)

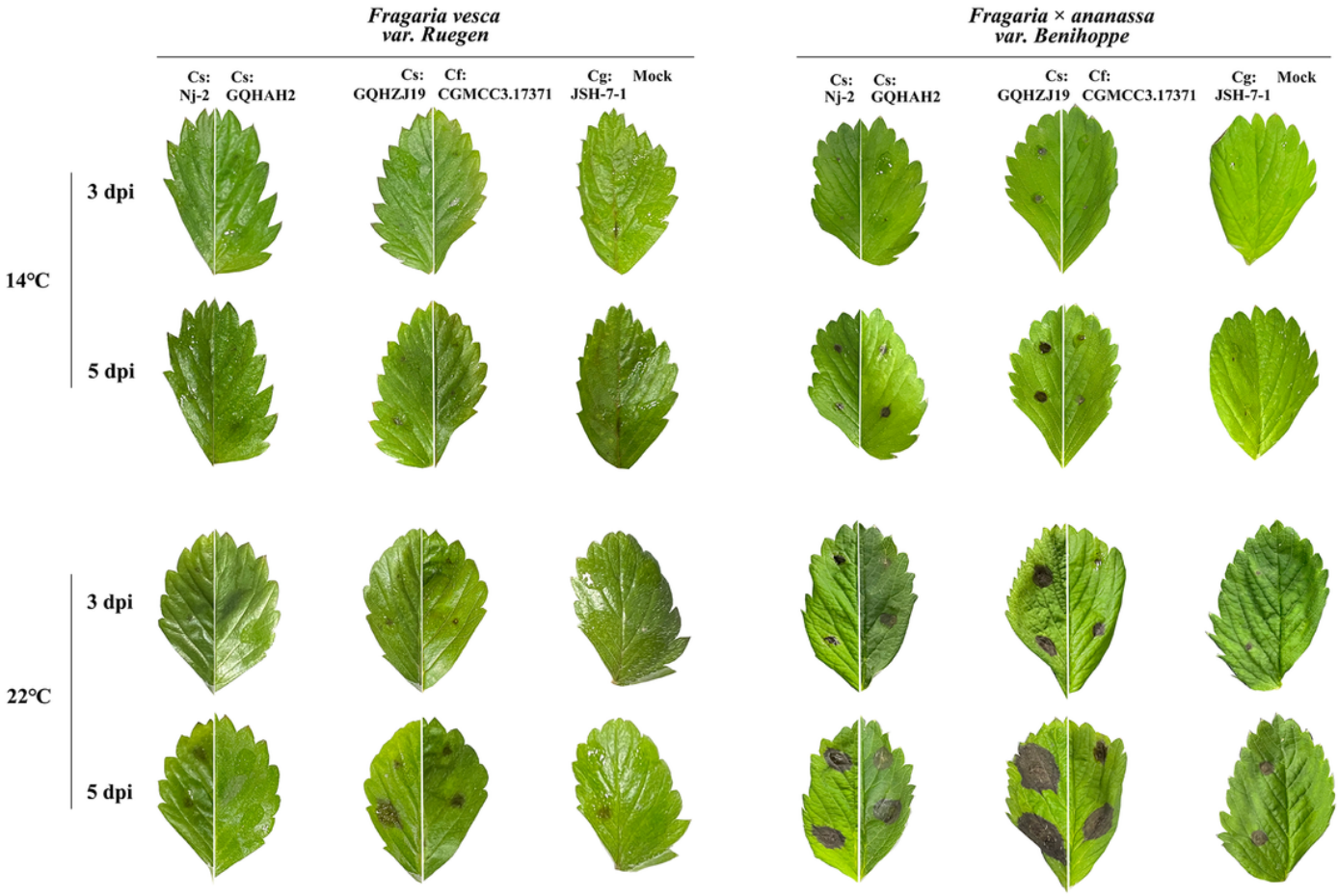


Figure 4

The severity of anthracnose necrosis on wounded detached strawberry leaves at 14°C and 22°C. Percentage of disease rating of all 42 inoculation spots per strain × temperature combination was scored according to a modified rating scale (Ji et al. 2004). Disease index (%) calculated following a previous formula (Zhang et al. 2020) was provided below each Bar stacking diagram for disease grade

Supplementary Files

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- Fig.S1.tif