https://doi.org/10.1590/1678-4499.20230082

PLANT PROTECTION
Article

Effects of temperature and wetness period on the monocyclic components of persimmon anthracnose

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Received: Apr. 14, 2023 | **Accepted:** Jul. 19, 2023

Section Editor: Robson Di Pierro

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How to cite: Moreira, R. R., Kowata-Dresch, L. S., Verbiski, F. S., Petermann, D. and May De Mio, L. L. (2023). Effects of temperature and wetness period on the monocyclic components of persimmon anthracnose. Bragantia, 82, e20230082. https://doi.org/10.1590/1678-4499.20230082

ABSTRACT: Anthracnose caused by *Colletotrichum horii* is one of the most important diseases in the persimmon tree. This study aimed to determine the influence of environmental variables on conidia germination, mycelial growth, and infection in persimmon fruit and shoots/twigs. The germination was quantified at 10, 15, 20, 25, and 30°C, with 6, 12, and 24 hours of wetness period. The effects of the same temperatures were also evaluated for mycelial growth, sporulation, and infection on 'Fuyu' fruit. The infection on shoots/twigs were quantified at 5, 10, 15, 22, 25, 27, and 35°C. The conidia germinated at all temperatures and wetness combinations, except at 10°C, with 6 hours of wetness. At 24 hours of wetness, the optimal temperature for conidia germination was 21.3°C. The optimal temperature for mycelial growth ranged between 21.7 to 24.3°C. The optimal temperature for lesion growth on fruit was 25.7°C; at 25°C, the incubation period was of two days, and the latent period lasted 12 days. On shoots/twigs, the temperature of 35°C anticipated in two days the onset of symptoms and 5-10°C delayed in eight-10 days the onset of symptoms in relation to temperature of 25°C. In nonlignified shoots, *C. horii* can cause symptoms from 15 to 35°C. In lignified twigs, *C. horii* was able to cause symptoms from 5 to 35°C. Because of the incidence and disease progression in most of climatic situations, our results reinforce the importance of monitoring and constantly removing diseased shoots, twigs, and fruits from the orchard to reduce the spread of the pathogen.

Key words: *Colletotrichum horii, Diospyros kaki*, monocycle.

INTRODUCTION

Persimmon (*Diospyros kaki* Thunb.) is a deciduous fruit tree, native from East Asia and widely cultivated in the world (Bassanezi and Amorim 1997, Badenes et al. 2003, Zhang 2008). In Brazil, persimmon tree was considered a rustic plant that did not require frequent phytosanitary treatments to obtain high yields. However, since 2006, reports are increasing in Paraná state regarding the high severity of anthracnose in the orchards (Blood et al. 2015).

Persimmon anthracnose is caused by the fungus *Colletotrichum* spp., including *C. horii*, *C. siamense* and *C. nymphaeae* in Korea (Kwon and Kim 2011, Kwon et al. 2013, Hassan et al. 2018, Hassan et al. 2019); *C. horii* and *C. karstii* in China (Wang et al. 2016, Xie et al. 2010, Zhang 2008); *C. acutatum* in the United States (Williamson and Sutton 2010); *C. gloeosporioides* in Spain (Palou et al. 2015), *C. horii* in Japan and New Zealand (Xie et al. 2010); and *C. horii, C. fructicola, C. nymphaeae, C. asianum* and *C. melonis* in Brazil (May De Mio et al. 2015, Carraro et al. 2019). Therefore, worldwide, the species *C. horii* is the main causal agent of persimmon anthracnose (Carraro et al. 2022).

The disease can affect leaves, shoots/twigs, and fruit. On leaves, the lesions appear on veins or nearby them, generally from the apex in the abaxial side, and the coalescence of these lesions can cause leaf drop. In shoots/twigs, the lesions are depressed and dark, and they can cause twig dieback or remain as cankers as the twigs age. In the fruit, the typical anthracnose lesions appear as small well-defined spots, depressed, dark brown to black, being able to develop and reach larger areas

of the fruit pulp. Under conducive environmental conditions, conidia is observed in concentric masses of gelatinous pink coloration. Infection of the pathogen can also occur by the flower, and, consequently, the developing fruit may present latent infections that can lead to a severe and premature fruit fall (Carraro et al. 2022, Dolinski et al. 2022).

In general, some factors favour the development of the disease, such as warm temperatures and humidity, mainly night temperatures between 20 to 25°C, and also inadequate fertilization and prolonged rainy period in the warmer months, usually at the beginning of fruiting (Dolinski et al. 2022). Quantification of the environmental factors that affect the processes of infection and colonization by the pathogen is essential to understand epidemics (Kranz and Hau 1980). These studies can be conducted in controlled conditions, and the data can be used to explain the development of epidemics in the field (Rotem 1988).

Therefore, this article aimed to study the favourable environmental conditions for pre-penetration and infection of *C. horii* on 'Fuyu' persimmon fruit and on 'Fuyu' persimmon nonlignified shoots and on lignified twigs, providing an epidemiological basis for future studies on this pathosystem.

MATERIAL AND METHODS

Colletotrichum horii isolates were previously identified (May De Mio et al. 2015) (Table 1). For the experiments, the fungus was cultured in Petri dishes (11.5-cm diameter) containing potato dextrose agar (PDA) amended with lactic acid (1 mL·L^{-1}), incubated at 20 ± 2°C for five days in the dark.

Table 1. *Colletotrichum horii* isolates from persimmon fruit and twig.

Isolate	Species[a]	Collection data					Information
		Year	Municipality	State	Cultivar	Plant part	
EMCo07	*C. horii*	2007	Lapa	Paraná	Kakimel	Twig	Organic system
EMCo11	*C. horii*	2009	Bocaiúva do Sul	Paraná	Kakimel	Fruit	Organic system
EMCo12	*C. horii*	2008	Quatro Barras	Paraná	Fuyu	Twig	No information
EMCo13	*C. horii*	2008	Campo Largo	Paraná	Kakimel	Twig	Organic system
EMCo14	*C. horii*	2007	Quatro Barras	Paraná	Fuyu	Twig	No information

[a]Single-spore isolates were molecularly identified as *Colletotrichum horii* (May De Mio et al. 2015). The isolates EMCo07, EMco11, EMCo13 and EMCo14 were renamed to DkPR11-07, DkPR09-11, DkPR10-13, DkPR10-14, respectively, and confirmed by multilocus analyses (Blood et al. 2020).

The influence of temperature and wetness period on conidia germination

Three 20-µL droplets from a freshly prepared conidia suspension (10^4 conidia·mL^{-1} of isolate EMCo14) were placed equidistant on a polystyrene Petri dish (5 cm in diameter). Three dishes per treatment were used, as well as three subsampling (droplets) within the replicate. These Petri dishes were placed without the lid in a plastic box containing filter paper moistened with 20 mL of sterile distilled water and maintained at 10, 15, 20, 25 and 30°C and wetness period (WP) of 6, 12 and 24 hours, in the dark. The germination process was interrupted with the addition of 20 µL of lactoglycerol on the fungal droplet. The germination was assessed for 100 conidia per droplet, and the conidia were considered germinated when the germ tube length was twice the width of the conidia.

Three temperatures (those with high percentages of germinated conidia) were selected and used in a similar experiment with discontinuous WP:

- Six hours WP + 18 hours without WP;
- Six hours WP + 12 hours without WP + six hours WP;
- 12 hours WP + 12 hours without WP;
- 24 hours WP.

The Petri dishes were opened and placed in a plastic box containing filter paper moistened with 20 mL of sterile distilled water, during the initial WP. The samples were maintained in a bio-oxygen demand (BOD) chamber. The plastic dishes were removed from the plastic boxes and placed under a laminar flow hood for 30 minutes, even after the droplet of the suspension was visibly dry. The plastic dishes were closed and remained outside of the plastic box during the predetermined dry period (without WP) in the BOD chamber. The wetness was re-established by placing 50 µL droplets of sterile distilled water upon the conidia, and the open Petri dishes were once more placed in the BOD chamber for the remaining time. A randomized block design was used. The three droplets in each dish (three dishes) for each combination of temperature and WP provided nine replicates in total per treatment.

The influence of temperature on mycelial growth

The five *C. horii* isolates listed in Table 1 were cultured in Petri dishes containing PDA amended with lactic acid (1 mL·L^{-1}). The Petri dishes were incubated at 20 ± 2°C for five days in the dark. Mycelial plugs from these dishes (5 mm in diameter) were transferred to the center of Petri dishes containing PDA amended with lactic acid (1 mL·L^{-1}). The Petri dishes were incubated at 10, 15, 20, 25 and 30 ± 2°C in a completely randomized design with five replications (dishes) per temperature. The mycelial growth was assessed by two perpendicular measures of fungal colony diameters on the third, sixth, and nineth day after incubation. Additionally, the day that sporulation began was identified by the presence of orange color on the upper and/or lower side of the colony, with the aid of a stereoscopic microscope. On this day, the structures of the fungus were collected for the preparation of microscope slides and confirmation of the presence of *C. horii* spores.

The influence of temperature on the monocyclic components on fruit

Persimmon fruit cv. 'Fuyu' were collected in a commercial orchard located in Bocaiuva municipality, Paraná state, Brazil. The fruit were disinfested with 1% sodium hypochlorite for 2 minutes and rinsed with sterile water. The fruit samples were placed in plastic boxes containing wet filter paper, and a wound on the equatorial area was made on the surface with a histological needle. The fruit were inoculated by placing a 10 µL droplet of conidia suspension (10^4 conidia·mL^{-1} of isolate EMCo14) on the wound. The inoculated fruit were incubated at 10, 15, 20, 25 and 30°C, under continuous humidity and darkness. The assessment of disease incidence and lesion diameter started two days after inoculation and continued on alternate days until the 16th day. For incidence, the percentage of fruit with any symptoms of the disease was calculated in relation to the total number of fruit. For severity, the longitudinal and transverse diameters of the lesions were measured (mm), and the mean was calculated to obtain the lesion size. To calculate the incubation and latency periods, the number of days for symptom onset and lesion sporulation, respectively, were considered in 50% of the fruit of each treatment. A completely randomized design was used, with each fruit being one replicate with 10 replicates per temperature, totaling 50 fruit in the experiment.

The influence of temperature on the monocyclic components on shoots/twigs

Four experiments were carried out, two with nonlignified shoots and two with lignified twigs. Persimmon shoots/twigs cv. 'Fuyu' with 10-cm length were collected in an experimental orchard located in Curitiba municipality, Paraná state, Brazil. The shoots/twigs were disinfested with 1% sodium hypochlorite for 2 minutes and rinsed with sterile water. The shoots/twigs samples were placed in plastic boxes containing wet filter paper, and a wound was made on the shoot/twig surface with a histological needle. The shoots/twigs were inoculated with the isolate EMCo14 by placing a 4-mm diameter mycelial plug on the wound.

The inoculated nonlignified shoots were incubated in the dark at 5, 10, 15, 22, 25, 27 and 35°C, under continuous humidity. The inoculated lignified twigs were incubated in the dark at 5, 10, 15, 20, 25, 30 and 35°C, under continuous humidity. The assessment of disease incidence and lesion length started one day after inoculation and continued every

day until the eighth day for nonlignified shoots and until the 22th day for lignified twigs. For severity, the lesion length was measured (mm) at the eighth and the 22nd day after inoculation for nonlignified shoots and lignified twigs, respectively. To calculate the incubation period, the number of days for symptom onset was considered in 50% of the shoots/twigs of each treatment. A completely randomized design was used, with each shoot/twig being one replicate with 25 replicates per temperature, totaling 175 shoots/twigs in each experiment.

Data analyses

The mycelial growth data were used to calculate the area under the mycelial growth (AUMG) for each treatment by trapezoidal integration, and the diameters of anthracnose lesions on fruit were used to calculate the area under the disease progress curves of severity (AUDPCS) for each treatment by trapezoidal integration (Shaner and Finney 1977). Beta-generalized model (Bassanezi et al. 1998) was fitted to data of spore germination (in percentage), AUMG (mm^2) and AUDPC (mm^2) (Eq. 1):

$$Y(T) = Yopt * \frac{\left[\frac{T - Tmin}{Topt - Tmin}\right]^{b3(Topt - Tmin)}}{Tmax - Topt} * \left[\frac{Tmax - T}{Tmax - Topt}\right]^{b3} \quad (1)$$

where: T: the temperature; Y: the monocyclic component considered; Y_{opt}: the value of germination (%); AUMG or AUDPCS at the optimal temperature; T: the temperature (°C); T_{min}: the minimum temperature; T_{opt}: the optimal temperature; T_{max}: the maximum temperature; B_3: the amplitude of the curve in its asymptotic range.

The analyses were performed using the R software (R Development Core Team 2016).

For the analysis of the discontinuous WP study, the tests of mean separation were determined by analysis of variance (F test), with arcsine transformation of the percent germination data. The means were compared with Tukey's test ($p > 0.05$).

Using the data of anthracnose incidence in shoots/twigs, a survival analysis was performed, considering the probability that shoots/twigs would remain symptomless over time. Data from the two experiments with shoots and from the two experiments with twigs were analyzed together as they did not present statistical differences. Kaplan-Meier curves were constructed to assess the likelihood of onset symptoms occurrence, and the Cox semi-parametric model was adjusted to compare the temperatures. The analyses were performed using the R software (R Development Core Team 2016), and the package 'survival' was used for survival analyses.

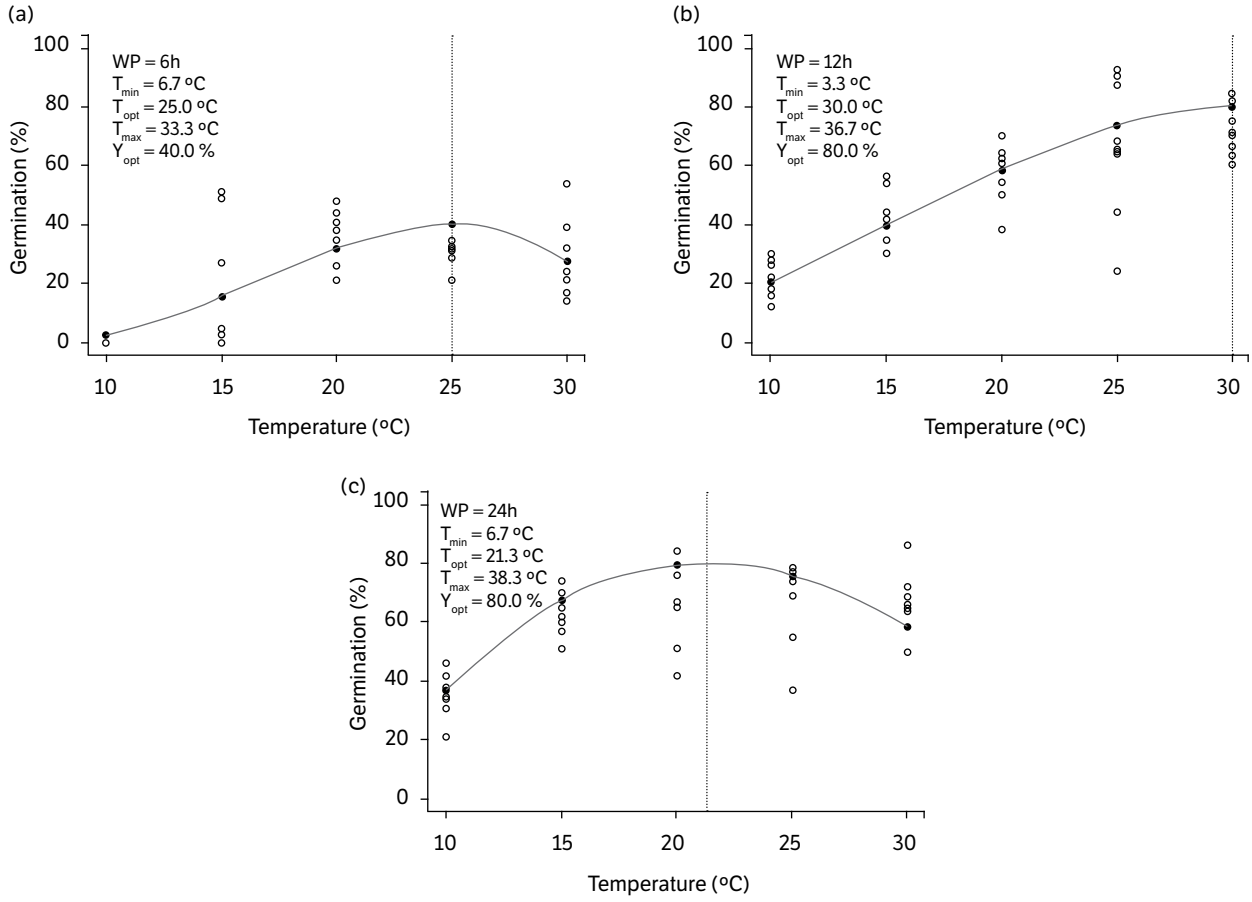
The averages of anthracnose lesion length on shoots/twigs were compared between temperatures by non-parametric Kruskal-Wallis' test for multiple comparison of treatments using R software (R Development Core Team 2016) and then added to the package 'agricolae' for an additional test to analyze which treatments are different from each other.

RESULTS

The influence of temperature and wetness period on conidia germination

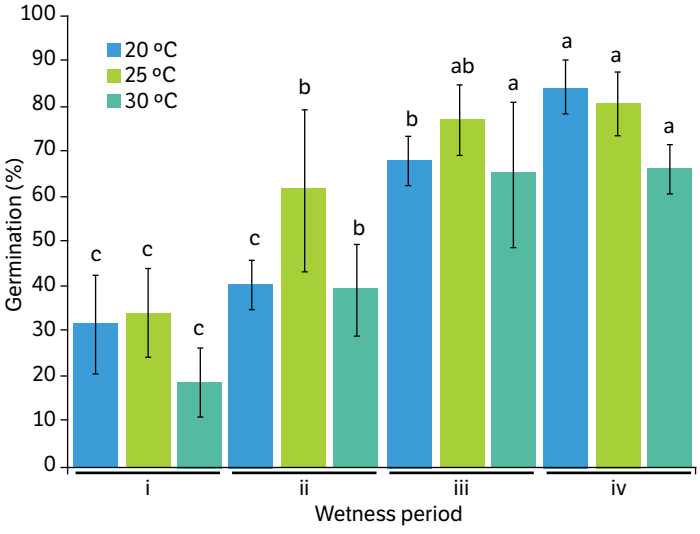
The conidia of EMCo14 isolate germinates at all temperatures, except at 10°C with 6 hours of wetness. The germination increased as the given period of wetness was longer, not exceeding 40% of germination at 6 hours of wetness and reaching 80% of germination at 12 and 24 hours of wetness (Fig. 1).

At 20°C, the conidia germination was significantly higher in a 24 hours of continuous WP when compared to the other wetness conditions. At 25 and 30°C, the conidia germination was similar in the wetness of 24 and 12 hours and significantly higher when compared to the other wetness conditions, in which the conidia remained only 6 hours with wetness and 12/18 hours without wetness (Fig. 2).



Y_{opt}: optimal percentage of conidia germination.

Figure 1. Minimum (T_{min}), optimal (T_{opt}) and maximum (T_{max}) temperatures (˚C) for conidia germination (%) in different wetness period (WP) of *Colletotrichum horii* isolate EMCo14 from persimmons and adjustments on the generalized Beta distribution according to Bassanezi et al. (1998). White circles represent the repetitions of each tested temperature; black circles were clear-cut by the statistical analysis used.



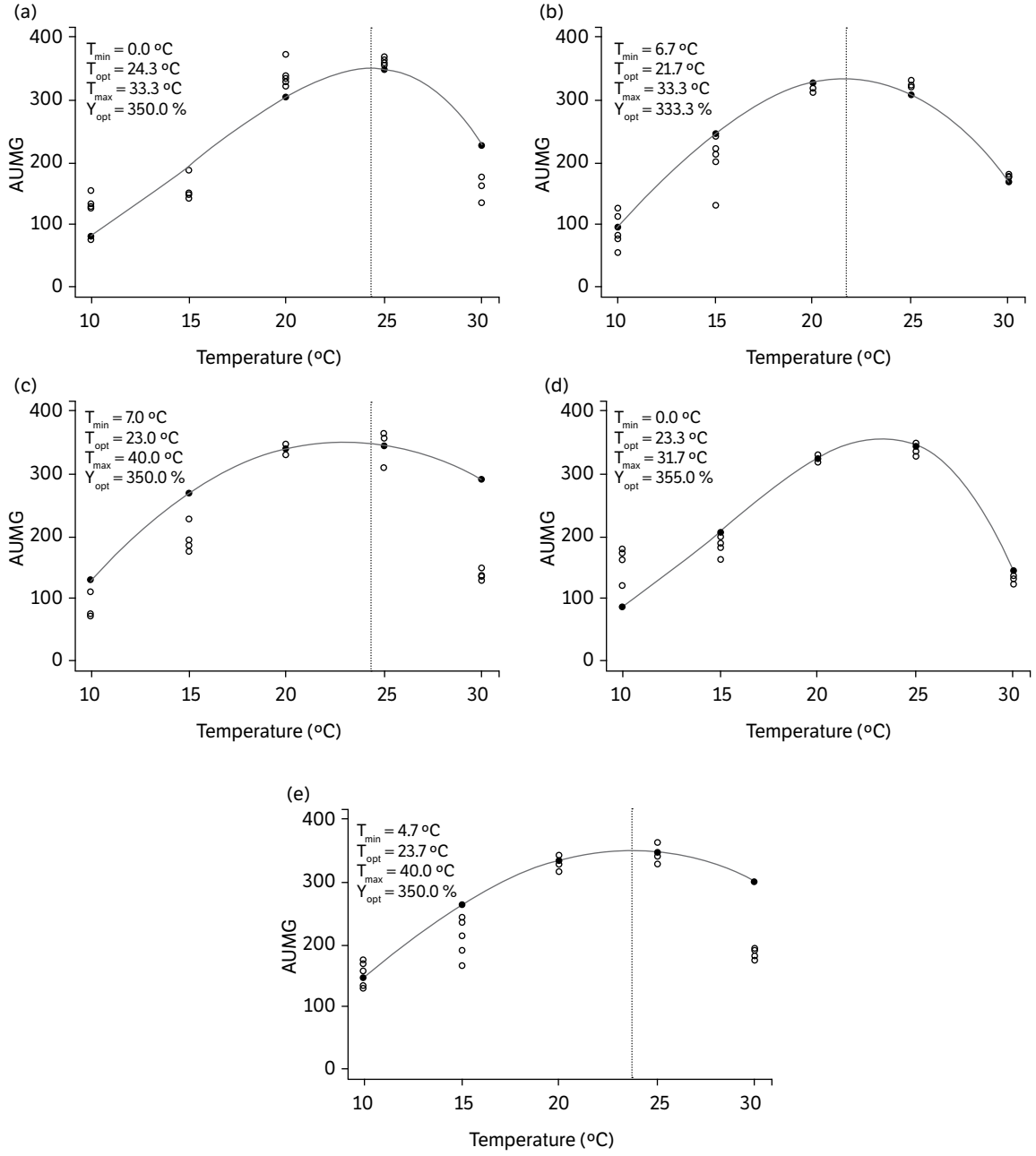
*Columns followed by the same latter are not different according to Tukey's test ($p < 0.05$), statistical analyses between WP at each temperature; coefficients of variation (%): 17.09 (20˚C), 23.67 (25˚C) and 29.16 (30˚C); bars indicate the standard deviation of the mean.

Figure 2. Conidia germination (%) of *Colletotrichum horii* isolate EMCo14 from persimmon under different temperatures (20, 25 and 30˚C) and discontinuous wetness period (WP) conditions: (i) six hours WP + 18 hours without WP ; (ii) six hours WP + 12 hours without WP + six hours WP ; (iii) 12 hours WP + 12 hours without WP , and (iv) 24 hours WP.

The influence of temperature on mycelial growth

The minimum, optimum and maximum temperatures, as well as the maximum AUMG, varied among the different *C. horii* isolates. The minimum temperature for mycelial growth varied from 0 to 7°C, the optimal temperature from 21.7 to 23.7°C, the maximum temperature from 31.7 to 40°C, and the maximum AUMG ranged from 333.3 to 355 mm^2 (Figs. 3a–3e).

All isolates sporulated at all tested temperatures, varying only the time, in days, for sporulation. The shortest time for colony sporulation was three days, recorded at 20 and 25°C for most of the isolates, except for isolate EMCo13, which sporulated at the sixth day at 25°C. In general, at 10°C, the colonies of all isolates took longer to sporulate, from six to nine days (Table 2).



Y_{opt}: optimal AUMG.

Figure 3. Minimum (T_{min}), optimal (T_{opt}) and maximum (T_{max}) temperatures (°C) for area under mycelial growth (AUMG) of *Colletotrichum horii* isolates from persimmons and adjustments on the generalized Beta distribution according to Bassanezi et al. (1998). White circles represent the repetitions of each tested temperature; black circles were clear-cut by the statistical analysis used. (a) EMCo07, (b) EMCo11, (c) EMCo12, (d) EMCo13, (e) EMCo14.

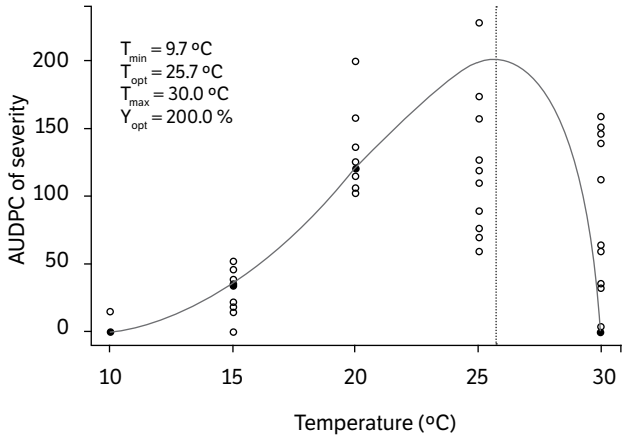
Table 2. Days for colony sporulation at different temperatures of *Colletotrichum horii* isolates from persimmon fruits.

Temperature (˚C)	Days for colony sporulation				
	EMCo07[a]	EMCo11[a]	EMCo12[a]	EMCo13[a]	EMCo14[a]
10	6	7	6	9	6
15	6	6	9	6	6
20	3	3	3	3	3
25	3	3	3	6	3
30	6	6	6	6	3

[a]Single-spore isolates were molecularly identified as *Colletotrichum horii* (May De Mio et al. 2015). The isolates EMCo07, EMco11, EMCo13 and EMCo14 were renamed to DkPR11-07, DkPR09-11, DkPR10-13, DkPR10-14, respectively, and confirmed by multilocus analyses (Blood et al. 2020).

The influence of temperature on the monocyclic components

For the isolate EMCo14, the minimum temperature for AUDPCS was 9°C, the optimal temperature was 25.7°C, the maximum temperature was 30°C, and the maximum AUDPC was 200 mm (Fig. 4).



Y_{opt}: optimal AUMG.

Figure 4. Minimum (T_{min}), optimal (T_{opt}) and maximum (T_{max}) temperatures (˚C) for the area under disease progress curve of severity (AUDPC of severity) on 'Fuyu' persimmon fruits inoculated with *Colletotrichum horii* isolate EMCo14 from persimmons and adjustments on the generalized Beta distribution according to Bassanezi et al. (1998). White circles represent the repetitions of each tested temperature; black circles were clear-cut by the statistical analysis.

Fruit lesions were more frequent and larger in fruits inoculated and kept at temperatures from 15°C, reaching a maximum growth near the temperature of 25°C (Fig. 4). Time to onset of symptoms was eight days for inoculated fruit maintained at 15°C and was only two days for fruit maintained at 20, 25 and 30°C. No sporulation was observed in inoculated fruit maintained at low temperatures of 10 and 15°C. The latent period was 14 days for inoculated fruit maintained at 20°C and 12 days for fruit maintained at 25 and 30°C (Table 3).

Table 3. Incubation and latent periods for anthracnose in 'Fuyu' persimmon fruit inoculated with *Colletotrichum horii* isolate EMCo14 and maintained at different temperatures.

Temperature (˚C)	Incubation period (days)	Latent period (days)
10	-	-
15	8	-
20	2	14
25	2	12
30	2	12

- Unable to calculate (less than 50% of the fruits showed symptoms and signs).

On nonlignified shoots, the first symptoms appeared with seven days after inoculation (DAI) at 25°C. The temperature of 35°C anticipated the onset of the first symptoms that occurred five DAI. Temperatures of 5 and 10°C delayed the onset of the first symptoms, which was > 16 and 15 DAI, respectively (Table 4). In nonlignified shoots, *C. horii* can infect and expand its lesion from 15 to 35°C, and the length of anthracnose lesions increases significantly with increasing temperature. At temperatures of 5 and 10°C, anthracnose lesion was not developed on nonlignified shoots (Fig. 5a).

Table 4. Relative risk for the expression of anthracnose symptoms caused by *Colletotrichum horii* isolate EMCo14 on 'Fuyu' persimmon nonlignified shoot and lignified twigs estimated by Cox semi-parametric model. Followed by the 95% confidence intervals of the temperatures.

Treatment (˚C)	Incubation period (days)	Relative risk	95% CI	
			LL	UL
Persimmon nonlignified shoots				
25	7			
35	5	1.261	1.0177	1.5612*
27	7	1.131	0.9150	1.3976
22	5	1.192	0.9675	1.4698
15	7	0.979	0.7877	1.2171
10	15	0.227	0.1614	0.3197**
5	>16	0.000	0.000	inf**
Persimmon lignified twigs				
25	12	-	-	-
35	10	2.024	1.630	2.512*
30	12	0.585	0.461	0.741**
20	12	0.615	0.486	0.777**
15	12	0.689	0.548	0.866**
10	14	0.552	0.432	0.704**
5	22	0.293	0.194	0.442**

LL: the lower limits of the confidence interval for the relative risk; UL: the upper limits of the confidence interval for the relative risk; *temperatures at which the risk of earlier onset of symptoms is significantly higher compared to 25˚C; **temperatures at which the risk of earlier onset of symptoms is significantly lower compared to 25˚C; 95%CI: 95% confidence intervals.

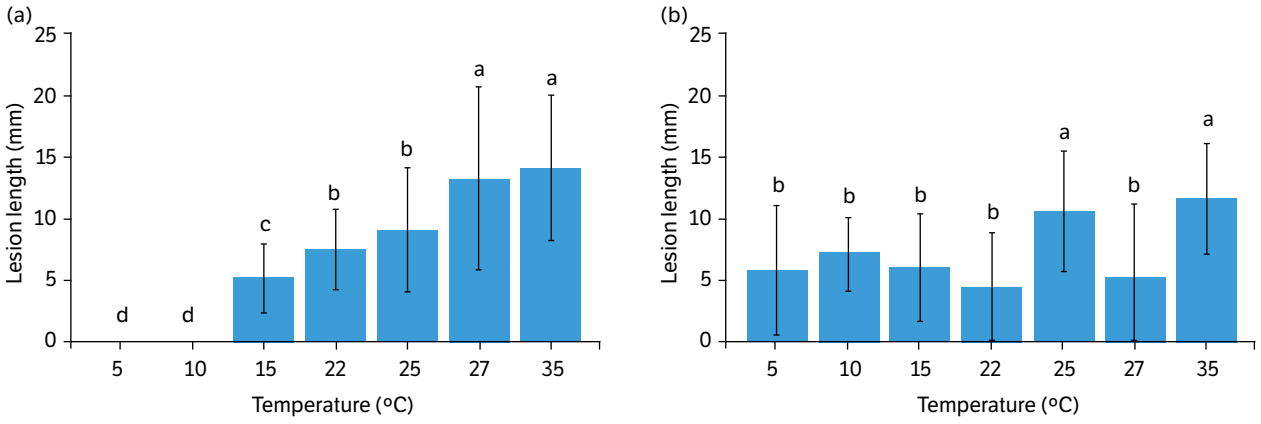


Figure 5. Anthracnose lesion length (mm) (a) on persimmon nonlignified shoots and (b) on persimmon lignified twigs at different temperatures eight and 22 days after inoculation with *Colletotrichum horii* isolate EMCo14, respectively. Columns followed by the same letter are not different according to Kruskal-Wallis' test. Bars indicate the standard deviation of the mean.

On lignified twigs, the first symptoms appeared with 12 DAI at 25°C. The temperature of 35°C anticipated the onset of the first symptoms that occurred 10 DAI. Temperatures of 5 and 10°C delayed the onset of the first symptoms, that was 22 and 14 DAI, respectively (Table 4). After the onset of anthracnose symptoms on lignified twigs, the length of the lesion was

highly variable at different temperatures. In lignified twigs, C. horii can infect and expand its lesion over a wide range of temperature, from 5 to 35°C. The temperatures of 25 and 35°C had significantly greater lesions (Fig. 5b).

DISCUSSION

This study demonstrated for the first time that *C. horii* and symptoms caused by this fungus in persimmon fruit and shoots/twigs in Brazil develop over a wide range of temperature, from 10 to 30°C for conidia germination, colony growth and symptoms on mature fruit, and from 15 to 35°C for symptoms on nonlignified shoots and from 5 at 35°C in lignified twigs. This study also showed that the optimal temperature for the *C. horii* development varies according to the WP and/or according to the isolate. Furthermore, the optimal temperature for the development of anthracnose symptoms and the time for the onset of symptoms may vary according to the age of the infected tissue.

In general, *C. horii* conidia germination increased with WP at all temperatures. Based on our data, the continuous wetness had more influence on germination than temperature. According to Arauz et al. (2010), an increase in *Pseudoperonospora cubensis* germination occurred with an increase in leaf WP at all tested temperatures (10, 15, 20, 25 and 30°C). The same results were observed in the present study and also in anthracnose infection (*Colletotrichum* spp.) on olive (*Olea europaea*, Moral et al. 2012).

Colletotrichum horii germinated, in different WP, in a wide range of temperatures. This fact was previously reported for *C. gloeosporioides* collected from guava, in which conidia germinated from 9.5 to 44.1°C and the optimum range was from 20 to 25°C, with more than 6 hours of wetness (Soares et al. 2008). Poltronieri et al. (2013) discussed that the conidia of *C. gloeosporioides* isolated from Juçara palm fruit (*Euterpe edulis* Mart.) germinated more often at a temperature of 28°C compared with temperatures of 20, 25, 32, and 35°C. These results are similar to those found by Maia et al. (2011), in which the highest percentage of germination occurred between 25 and 30°C.

The estimated temperature for mycelial growth of *C. horii* ranged from 0 to 40°C, depending on the isolate, and the optimal temperature for mycelial growth ranged from 21.7 to 24.3°C. High temperatures, above 35°C, prevent the growth of other species of *Colletotrichum*; for example, no mycelium growth of *C. gloeosporioides* from palm fruit was observed at 35°C (Poltronieri et al. 2013); and, higher temperatures, above 40°C inhibits mycelium growth of *C. gloeosporioides* from persimmon (Zhang and Hu 2004).

There are no reports of the optimal temperature for persimmon fruit infection by *Colletotrichum* species. Hassan et al. (2018) compared anthracnose lesion growth in persimmon fruit by *C. siamense* and by *C. horii*, but at only one temperature (25°C). We verified that the optimal temperature for persimmon fruit infection (25.7°C) was comparable with other reports of *Colletotrichum* spp. in other fruits. For example, in guava fruit inoculated with *C. gloeosporioides* and with *C. acutatum*, the maximum anthracnose severity, represented by lesion diameter, occurred at 25°C (Soares et al. 2008).

In the present study, the incubation period was two days in fruit inoculated with *C. horii* and kept at temperatures above 20°C. Zhang and Hu (2004) reported an incubation period of three days in persimmons (cv. Wuheshi) inoculated with *C. gloeosporioides* at 25°C. In the present study, the fruit lesions caused by *C. horii* did not sporulate at lower temperatures (10 and 15°C), and the latent period was from 12 to 14 days at temperatures above 20°C. However, the temperature of 30°C may stress and induce sporulation in the pathogen. King et al. (1997) found that the latent periods for *C. acutatum, C. gloeosporioides*, and *C. fragariae* infection on detached strawberries were dependent on temperature (5, 10, 15, 20, 25, 30, and 35°C) and ranged from two to three days at 25°C to six to 17 days at 5°C. In this case, the sporulation increased over time at temperatures of 15°C and above. Zhang and Hu (2004) reported a latent period of four days in persimmon fruit inoculated with *C. gloeosporioides* at 25°C. This large difference in the latent period compared to our work may be related to the inoculated *Colletotrichum* species (*C. gloeosporioides* × *C. horii*) and the inoculated cultivar (Wuheshi × Fuyu).

On shoots, Zhang and Hu (2004) reported the importance of pH between 4 and 8 for lesion development and also related that at 17 and 23°C lesions were observed after seven days, but below 17°C no symptoms were observed. Similarly, the present study showed that on nonlignified shoots, *C. horii* can infect and expand its lesion from 15 to 35°C and the symptoms

onset occurred after five–seven days, but no symptoms were observed at 5 and 10°C. Nevertheless, on lignified twigs, the range of temperature to the development of lesions caused by *C. horii* is greater, ranging from 5 to 35°C. Investigating why symptoms occur at 5 and 10°C in lignified twigs, but not on nonlignified shoots, is a challenge for future research. Anyway, the development of anthracnose lesions on shoots/twigs over a wide range of temperatures, and the survival of *Colletotrichum* spp. in one-year-old shoots of persimmon reported by Dolinski et al. (2022) highlight the importance of monitoring and constantly removing diseased shoots/twigs from the orchard to reduce the spread of the pathogen to other shoots/twigs and also to leaves, fruit, and flowers.

Research on pathogen development under controlled conditions can improve future studies of disease establishment risk, surveillance and eradication, climate change effect assessment, and, possibly, disease risk forecasting. The favorable conditions for *C. horii* infection and anthracnose development on fruit and on shoot/twig are easily found in the field in Brazilian regions with persimmon production. Therefore, this study can help to develop a daily index of environmental favorability for the disease or to minimize unnecessary sprays for control. Besides that, the present work presents an important step regarding the initial study of infection and lesion formation in persimmon fruit and shoots/twigs, unveiled important aspects of the aggressiveness of *C. horii*, and raised new questions for any future research.

CONCLUSION

The *C. horii* growth and the development of anthracnose lesions in persimmon fruit and shoots/twigs are favored by long periods of wetness and occur over a wide range of temperature. On fruit, *C. horii* can cause symptoms from 15 to 30°C, and the lesion diameter reaches a maximum close to 25°C. In nonlignified shoots, *C. horii* can cause symptoms from 15 to 35°C, and the lesion diameter significantly increases with increasing temperature. In lignified twigs, *C. horii* was able to cause symptoms from 5 to 35°C. Because of the incidence and disease progression in most climatic situations in which persimmon is planted, our results reinforce the importance of monitoring and constantly removing diseased shoots/twigs from the orchard to reduce the spread of the pathogen.

AUTHORS' CONTRIBUTION

Conceptualization: Kowata-Dresch, L. S. and May De Mio, L. L.; **Methodology:** Kowata-Dresch, L. S., Verbiski, F. S. and May De Mio, L. L.; **Investigation:** Moreira, R. R., Petermann, D., Kowata-Dresch, L. S., Verbiski, F. S. and May De Mio, L. L.; **Writing – Original Draft:** Moreira, R. R., Kowata-Dresch, L. S. and May De Mio, L. L.; **Writing – Review and Editing:** Moreira, R. R., Petermann, D. and May De Mio, L. L.; **Funding Acquisition:** May De Mio, L. L.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico
https://doi.org/10.13039/501100003593
Grant No. 306886/2021-9

ACKNOWLEDGMENTS

Not applicable.

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