ARTIGOS

Effect of temperature, wetness duration and cultivar on the development of anthracnose in guava fruits

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ABSTRACT

Soares-Colletti, A.R.; Lourenço, S.A. Effect of temperature, wetness duration and cultivar on the development of anthracnose in guava fruit**s.** ***Summa Phytopathologica,*** v.40, n.4, p.307-312, 2014.

The development of a large number of postharvest diseases is closely associated with fruit ripeness. Environmental conditions may affect both the pathogen development and the fruit ripening rate. The aim of this study was to determine the most favorable temperature and wetness duration to the development of anthracnose in guava fruits. Cultivars 'Kumagai' (white pulp) and 'Pedro Sato' (red pulp) were inoculated with a conidial suspension of *Colletotrichum gloeosporioides* and *C. acutatum* and incubated at constant temperature ranging from 10 to 35ºC and wetness duration of 6 and 24 hours. Disease severity and incidence were evaluated at every two days during 12 days. No infection occurred at 10 and 35ºC, regardless of the wetness duration. The optimum conditions for fruit infection were 26 and 27ºC for 'Kumagai' and 25 and 26ºC for 'Pedro Sato', adopting 24 hours of wetness. In general, the disease development in 'Kumagai' cultivar was more affected by the wetness period, compared to 'Pedro Sato'. Disease severity for 'Kumagai' fruits was maximal between 25 and 30ºC, depending on the *Colletotrichum* species. Regarding 'Pedro Sato', the mean diameter of lesions was greater in fruits stored at 20, 25 and 30ºC , compared to 'Kumagai' cultivar, depending on the wetness period and the species. The incubation period (between 6 and 7 days) and the latent period (between 8 and 10 days) were minimal at 30ºC. The data generated in this study will be useful either for the development of a disease warning system or for the increase in the shelf life of guavas in the postharvest.

Additional keywords: *Psidium guajava*, incidence, severity, incubation period, latent period, 'Kumagai', 'Pedro Sato'.

RESUMO

Soares-Colletti, A.R.; Lourenço, S.A. Efeito da temperatura, duração do molhamento e cultivares na anctrose em fruto da goiaba. ***Summa Phytopathologica,*** v.40, n.4, p.307-312, 2014.

O desenvolvimento de muitas doenças pós-colheita está intimamente associado ao amadurecimento dos frutos. As condições ambientais podem afetar tanto o desenvolvimento do patógeno quanto a taxa de amadurecimento dos frutos. O objetivo deste trabalho foi determinar as condições de temperatura e duração do período de molhamento mais favoráveis ao desenvolvimento da antracnose em frutos de goiaba. Frutos de goiaba das cultivares Kumagai (polpa branca) e Pedro Sato (polpa vermelha) destacadas foram inoculadas com suspensão de conídios de *Colletotrichum gloeosporioides* e *C. acutatum* e incubados sob temperaturas constantes variando entre 10 e 35ºC e molhamento contínuo de 6 e 24 horas. A severidade e incidência da doença foram avaliadas a cada dois dias durante 12 dias. Não houve infecção nas temperaturas de 10 e 35ºC, independentemente do período de molhamento. As condições ótimas para a infecção do fruto foram sob temperatura de 26 e 27ºC, para 'Kumagai' e de 25 e 26ºC, para 'Pedro Sato', com 24 horas de molhamento. De um modo geral, o desenvolvimento da doença na cultivar Kumagai foi mais afetado pela duração do período de molhamento, comparada com a 'Pedro Sato'. A severidade máxima da doença em frutos ocorreu entre 25 e 30ºC, dependendo da espécie de *Colletotrichum*, para 'Kumagai'. Para 'Pedro Sato' o diâmetro médio das lesões foi maior em frutos armazenados a 20, 25 e 30ºC do que na cultivar Kumagai, dependendo do período de molhamento e espécie. O período de incubação (6 e 7 dias) e o período de latência (8 e 10 dias) foram mínimos à 30ºC. Estas informações geradas nesse trabalho serão úteis tanto para o desenvolvimento de sistemas de aviso fitossanitário quanto para aumentar o tempo de vida de goiabas após a colheita.

Palavras-chave adicionais: *Psidium guajava*, incidência, severidade, período de incubação, período de latência, 'Kumagai', 'Pedro Sato'.

The guava fruit (*Psidium guajava* L.) is an excellent source of vitamins A, B and C, as well as of calcium, zinc, phosphorus and iron. Red guava can be used for fresh consumption and by the agricultural industry, and 'Pedro Sato' is one of the most important cultivars in Brazil (16, 28). White guava is used for fresh consumption, and 'Kumagai' is the main cultivar exported from Brazil (15).

Brazil is the world's largest producer of red guavas and the second producer of white guava. Despite its great production, Brazil is an insignificant exporter of guava *in natura* due mainly to the high perishability of the fruit in the postharvest (23, 14). Guava has a shelf life that can reach 3 to 5 days at room temperature. Postharvest damages, including postharvest diseases, are estimated at 20 to 40% (24, 8).

Anthracnose – one of the main postharvest diseases affecting guava fruit in Brazil (4, 17, 9) – has as causal agents *C. gloeosporioides* (Penz.) Sacc. and *C. acutatum* Simmonds. This disease affects both pre- and postharvest management of guava in Brazil, but most damages occur in the postharvest.

The infection can occur in the young fruit, in which the fungus remains quiescent after the formation of appressorium and penetration peg (19). However, symptoms are usually observed later on the ripening fruit. The fruit rot begins as a small, light brown, circular lesion. As lesions enlarge, they change to dark brown and form sunken depressions. After the enlargement of lesions, acervuli are produced in concentric circles around the infection site. Large amounts of conidia are produced in the acervuli on the fruit surface (21).

The weather variables such as temperature, relative humidity, rainfall and number of rainy days greatly influence the development of the disease. Under warmer temperature conditions, pathogens can grow rapidly and severe outbreaks of diseases are expected during the prolonged rainy season (20). The germination and appressorium formation rate is high in the range of 15 to 30°C for *C. gloeosporioides*, peaking at 25°C. *C. acutatum* is more sensitive to a variation in temperature than *C. gloeosporioides*, peaking at 20°C. The wetting period affects more *C. acutatum* than *C. gloeosporioides*, and germination was lower at 6 hours of wetting than at 12 and 24 hours (26).

The relationship between the dependence of temperature and wetness duration with the disease percentage, established under controlled conditions, cannot always be observed in the field. This is due especially to variations in temperature and wetness duration inside the orchard. It is also due to variables existing in the field and not present in controlled condition studies, like fruit in different stages and physiological conditions (6). However, knowledge of infection and colonization processes of *Colletotrichum* spp., as well as of the factors that influence such processes, is a pre-requisite for the development of effective control strategies, like a warning system based on weather conditions (10).

Given the importance of finding a feasible solution to reduce anthracnose incidence and to extend guava fruit quality in the postharvest and considering the scarce information about the favorable conditions to its development, the aim of this paper was to assess the effect of different wetness durations and incubation temperatures on the incidence and severity of anthracnose caused by *C. gloeosporioides* and *C. acutatum* in 'Kumagai' and 'Pedro Sato' guavas.

MATERIALS AND METHODS

Inoculum production

C. gloeosporioides and *C. acutatum* isolates used in this study were obtained by direct isolation in water-agar (15g agar, 500 mL water) from guava fruit showing anthracnose symptoms, collected from a producing orchard in Campinas City, in the region of São Paulo (22°54'20''S; 47°03'39''W). After incubation for three days at 25ºC and 12h photoperiod, the fungi were transferred to plates containing PDA (200g boiled potatoes, 20g dextrose and 15g agar) and maintained in a growth chamber under the same temperature and photoperiod conditions.

PCR amplification was used to identify isolates as *C. gloeosporioides* or *C. acutatum* using species-specific primers (18, 27, 13). The methodology used for PCR analysis was described by Adaskaveg & Hartin (1).

Conidia were produced by culturing both isolates on oat agar (40 g flour, 15 g agar, 1000 mL water) plates incubated at 25ºC and 12h photoperiod for 7 days. Immediately prior to inoculation, inoculum was prepared by adding 10 mL sterile distilled water to three Petri plates containing each isolate. Resultant suspensions were transferred to a sterile beaker. Inoculum concentrations were adjusted (using a hemocytometer) to 1 x 10^5 conidia mL^{-1}, for subsequent inoculations into 'Kumagai' and 'Pedro Sato' guava fruits.

Guava inoculation and incubation

'Kumagai' guava fruits were collected from a producing orchard in Campinas, SP, while 'Pedro Sato' guava fruits were obtained from the **Central Food Supply of Campinas S.A** (CEASA), also located in Campinas, SP. Fruits were surface disinfested with sodium hypochlorite solution (0.5 % commercial product for 5 min) and rinsed with running water. After drying, the fruits were placed in individual plastic pots containing a piece of moistened cotton. On each fruit, one wound (1 mm in diameter and 5 mm in depth) was made with a histological needle at the central area. The wounded sites were inoculated with 50 µL conidial suspension of each species (1 x 10^5 conidia mL^{-1}), using a micropipette. Control fruits were wounded and inoculated with sterile distilled water. Immediately after inoculation, the fruits were kept in growth chambers (BOD Incubator, Model EL 202) set at 10, 15, 20, 25, 30 and 35ºC, ±2°C, and 12 h photoperiod. The fruits were kept under wet chamber conditions for 6 and 24 h. After these periods, the moistened cotton was removed and the lids of pots were opened.

At the end of the incubation period, disease severity was assayed for each fruit by measuring the average lesion diameter on days 2, 4, 6, 8, 10 and 12 after inoculation. Two diameters were measured at the right angles across lesions on the surface of infected fruits.

Data are expressed as the means of lesion diameters. Fruit incidence was evaluated by counting the number of infected fruits from the total number of fruits at every 2 days. Disease incidence progress curves were analyzed and compared by calculating the area under the disease incidence progress curve (AUDPC) for each cultivar. Incubation period, defined as the time from inoculation to when 50% of the fruits presented symptom, and latent period, defined as the time from inoculation to when 50% of the fruits presented fungal sporulation, were determined at the end of the experiments.

Experimental design was completely randomized, including two *Colletotrichum* species, two cultivars, 10 fruits, two wetness periods, and 6 temperatures, totaling 600 fruits per experiment, and considering control treatment. The order of tested temperatures, as well as the incubator used for each temperature, was assigned at random. The experiment was conducted twice.

Statistical analysis

The effects of wetness duration and temperature on fruit incidence were evaluated based on the disease incidence (%) and the areas under the disease incidence progress curve (AUDPC), calculated by trapezoidal integration (5).

Non-linear regression models were used to describe the relationship between the AUDPC and the temperature. The tested models were generalized β model and quadratic model. The generalized β model was selected because it provided a good fit for all combinations of isolates and cultivars. The generalized β model (11) uses the following equation: $Y=(b_1*((t-b_2)^b_3)*((b_4-t)^b_5))$, in which Y corresponds to the AUDPC, t corresponds to the temperature (ºC), b_2 and b_4 are the minimum and maximum temperatures, respectively, b_1 and b_3 and b_5 are the model parameters. The relationship between mean lesion

diameter (12 days after inoculation) and temperature was also analyzed by the generalized β model, using the following equation: $Y=(b_1*((t-b_2)^b_3)*((b_4-t)^b_3))$, in which Y corresponds to the severity (lesion diameter in cm), t corresponds to the temperature (ºC), b_2 and b_4 are the minimum and maximum temperatures, respectively, b_1 and b_3 and b_5 are model parameters. *T*-test was used to compare the experiments, based on the model parameters. Data on final lesion diameter (12 days after incubation) were subjected to analysis of variance and means were compared by Tukey's multiple test ($p<0.05$). Non-linear regression analysis was used to evaluate the relationship between the incubation and latent periods and the temperature. The Power Law model was used for data that presented high values at low temperatures and low values at high temperatures. It is described by the equation $y=at^b$, where y is the incubation or latent period (days), t is the temperature (ºC) and a and b are the model parameters (7). All data analyses were performed by using STATISTICA software (version 7.0, StatSoft Inc., Tulsa, Oklahoma).

RESULTS AND DISCUSSION

Temperature significantly influenced guava infection by both fungal species. The disease did not develop at 10ºC and 35ºC, regardless of the wetness period, for both cultivars, after 12 days of incubation ($p\leq0.05$) (Figure 1). The first symptoms were observed at four days after inoculation for fruits stored at 30ºC and both wetness periods.

The highest disease incidence (100%) occurred at 25 and 30ºC and 6 hours of wetness duration for both *Colletotrichum* species at 10 days after inoculation in 'Pedro Sato' and at 12 days in 'Kumagai' guavas (data not shown). The AUDPC showed a significant correlation between disease incidence and temperature. There was an increase in AUDPC with the temperature increase until 30 ºC for 'Kumagai' guava. For 'Pedro Sato', the AUDPC was similar from 20 to 30ºC and lower at 15ºC (Figure 1).

The optimal conditions for fruit infection were 27ºC and 26ºC for *C. gloeosporioides* and *C. acutatum*, respectively, in 'Kumagai' guavas, 26ºC and 25ºC for *C. gloeosporioides* and *C. acutatum*, respectively, in

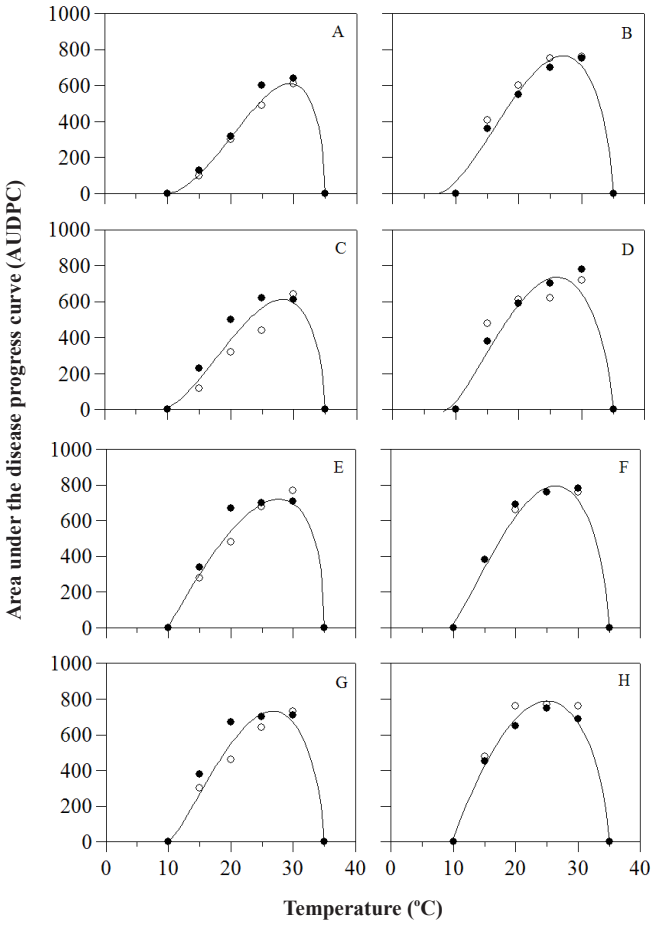

Figure 1. Area under disease progress curves (AUDPC), caused by *Colletotrichum gloeosporioides* (A, B, E and F) and *C. acutatum* (C, D, G and H) in 'Kumagai' (A, B, C and D) and 'Pedro Sato' (E, F, G and H) guava fruits incubated at different temperatures and wetness periods (6 hours – A, C, E and G; 24 hours – C, D, F and H). Black circles represent mean data from the first experiment and white circles represent mean data from the second one

Table 1. Coefficients of determination (R^2), parameters and optimum temperature (Topt) according to generalized β model [$Y=(b_1*((t-b_2)^b_3)*((b_4-t)^b_5))$, in which Y corresponds to the AUDPC, t corresponds to the temperature (ºC), b_2 and b_4 are the minimum and maximum temperatures (°C), respectively, b_1, b_3 and b_5 are model parameters] fitted to the area under progress curve (AUDPC) of anthracnose (*Colletotrichum gloeosporioides* and *C. acutatum*) observed under 6 or 24 hours of wetness duration in 'Kumagai' and 'Pedro Sato' guava fruits.

Variables	Wetness duration	b_1	b_2	b_3	b_4	b_5	R^2	Optimal T(°C)
'Kumagai' guava								
C. gloeosporioides								
AUDPC	6 hours	1.15	9.88	1.81	35.00	0.52	0.99	29
AUDPC	24 hours	1.40	7.15	1.65	35.00	0.66	0.97	27
C. acutatum								
AUDPC	6 hours	1.59	9.06	1.64	35.00	0.58	0.94	28
AUDPC	24 hours	2.61	8.57	1.42	35.00	0.72	0.94	26
'Pedro Sato' guava								
C. gloeosporioides								
AUDPC	6 hours	14.88	9.90	1.05	35.00	0.43	0.97	28
AUDPC	24 hours	5.20	9.35	1.28	35.00	0.65	0.98	26
C. acutatum								
AUDPC	6 hours	4.86	10.00	1.30	35.00	0.64	0.95	27
AUDPC	24 hours	11.58	9.76	1.00	35.00	0.65	0.98	25

'Pedro Sato' guavas, and 24 hours of wetness duration in both cultivars. The generalized β model equation parameters fitted to the AUDPC (Table 1). According to *t*-test, no variation was observed between experiments 1 and 2 ($p \leq 0.05$).

Results indicate that both *Colletotrichum* species prefer warm environments but can grow within a wide range of temperature and wetness duration. In general, at the corresponding optimal temperatures, no significant differences in the disease development occurred between *C. gloeosporioides* and *C. acutatum*. Depending on the fruit, *Colletotrichum* species can develop differently. Strawberry fruits inoculated with different *Colletotrichum* species showed that *C. fragarie* caused more severe symptoms than *C. acutatum*, which in turn caused more severe symptoms than *C. gloeosporioides* (25).

The disease incidence was more affected by wetness duration in 'Kumagai' than in 'Pedro Sato' fruits. The AUDPC for 'Kumagai' guava was greater with 24 hours of wetness duration than with 6 hours, at all temperatures evaluated for both *Colletotrichum* species (Figure 1). Similar results were observed for 'Safeda' guava. The incidence of disease caused by *Colletotrichum gloeosporioides* was rather reduced (less than 10%) when fruits were subjected to wetness durations of 6 hours or less, compared to 24 hours of wetness duration (20).

The maximum disease severity, represented by lesion diameter, occurred at 25ºC, regardless of the wetness duration, for 'Kumagai', considering both *Colletotrichum* species (Table 2). Lesion diameter in 'Kumagai' was greater at 15 and 20 ºC with 24 hours of wetness duration, compared to 6 hours. For 'Pedro Sato' guava, mean lesion diameters were greater in fruits stored at 20, 25 and 30ºC, depending on the wetness period and the species ($p \leq 0.05$) (Table 2). Mean lesion diameter was greater in 'Pedro Sato' than in 'Kumagai' guavas stored at 15 and 20ºC. At 25ºC, mean lesion diameters were similar in both cultivars, and at 30ºC the mean lesion diameter was greater in 'Kumagai' guava with 24 hours of wetness.

Since germination and infection processes demand long periods, wetness duration also influences the infection success. The duration necessary for infection varies with the temperature. Usually, a longer wetness period is needed for the infection to establish at cooler temperatures, as germination and infection are generally accelerated under warmer conditions (22).

The disease development was significantly different between guava cultivars. 'Kumagai' guavas stored at 15°C presented lower disease incidence and lesion diameter and showed longer incubation and latent periods than 'Pedro Sato' guavas. Anthracnose developed better and more rapidly in 'Kumagai' guavas at higher temperatures and longer wetness periods, compared to 'Pedro Sato'.

The colonization process of *C. gloeosporioides* in guavas is intracellular hemibiotrophic (3, 19), and the penetration can occur through wounds, natural openings or directly via appressorium (12). The ripening process of red pulp guava seems not to depend on the storage temperature. During the storage period, there is a reduction in firmness and weight, and an increase in ºBrix. Fresh mass loss is one

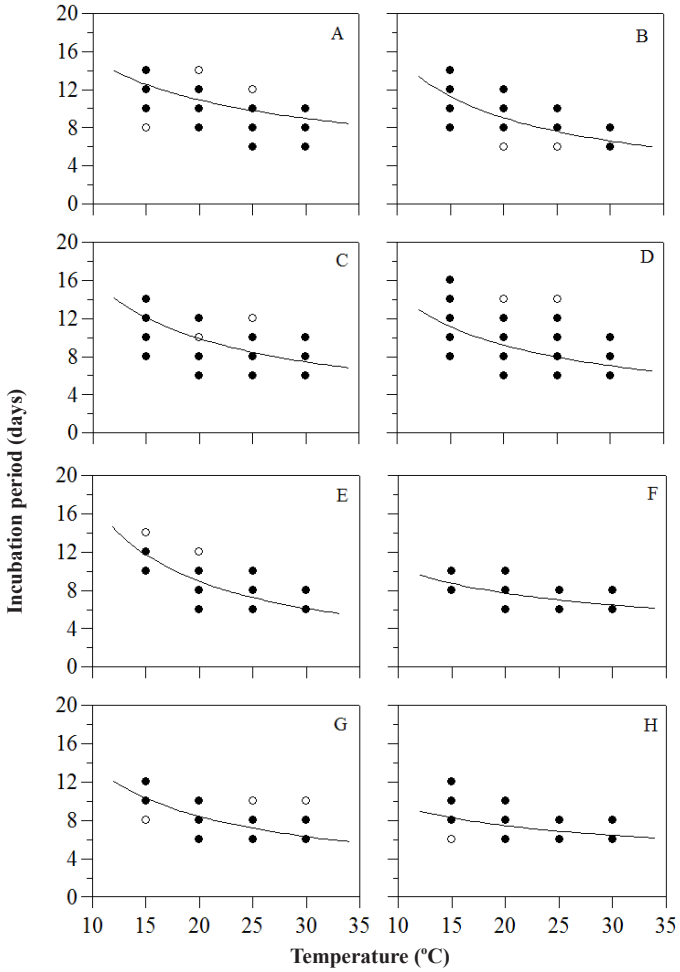

Figure 2. Incubation periods of *Colletotrichum gloeosporioides* (A, B, E and F) and *C. acutatum* (C, D, G and H) in 'Kumagai' (A, B, C and D) and 'Pedro Sato' (E, F, G and H) guava fruits stored at different temperatures and wetness periods (6 hours – A, C, E and G; 24 hours – C, D, F and H). Black circles represent data from the first experiment and white circles represent data from the second one

Table 2. Lesion diameter caused by *Colletotrichum gloeosporioides* (*C.g.*) and *C. acutatum* (*C.a.*) in 'Kumagai' and 'Pedro Sato' guava fruits stored at different temperatures and wetness periods, 12 days after inoculation.

Temperature (°C)	**Lesion diameter (cm)**							
	'Kumagai'				**'Pedro Sato'**			
	C.g.		***C.a.***		***C.g.***		***C.a.***	
	6h	**24h**	**6h**	**24h**	**6h**	**24h**	**6h**	**24h**
10	0.0 A d	0.0 A e	0.0 A f	0.0 A f	0.0 A e	0.0 A e	0.0 A d	0.0 A d
15	0.6 E d	1.4 CD d	1.1 CD e	2.0 B de	1.9 BC d	2.3 AB cd	2.4 AB c	2.6 A bc
20	2.1 BC bc	2.6 AB bc	2.0 Cc d	3.1 A bc	2.8 AB bc	3,1 A a	3.1 A b	2.9 A ab
25	3.1 B a	4.0 A a	3.4 AB a	4.2 A a	3.3 A ba	3.3 AB a	3.6 AB a	3.2 B a
30	2.9 B a	3.0 B b	2.4 B bc	4.0 A ab	3.0 B ab	2.5 B bc	3.1 B b	2.5 B c
35	0.0 A d	0.0 A e	0.0 A f	0.0 A f	0.0 A e	0.0 A e	0.0 A d	0.0 A d

*Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ according to Tukey's test ($p \leq 0.05$). Data represent the mean of two experiments.

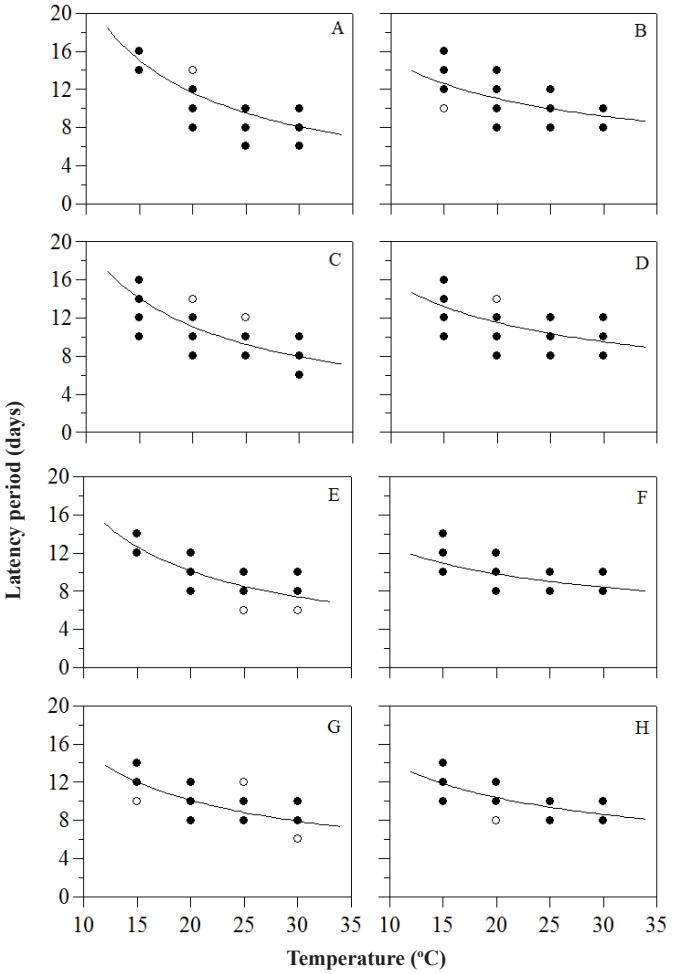

Figure 3. Latent periods of *Colletotrichum. gloeosporioides* (A, B, E and F) and *C. acutatum* (C, D, G and H) in 'Kumagai' (A, B, C and D) and 'Pedro Sato' (E, F, G and H) guava fruits stored at different temperatures and wetness periods (6 hours – A, C, E and G; 24 hours – C, D, F and H). Black circles represent data from the first experiment and white circles represent data from the second one

of the main problems during the fruit storage, since water loss leads to tissue softening, making fruits susceptible to deterioration (2).

The minimum incubation period for both species was 6 and 7 days for 'Pedro Sato' and 'Kumagai' fruits, respectively, kept at 30ºC and 24 hours of wetness duration (Figure 2). The minimum latent period for both fungal species was 9 days in 'Kumagai' guavas and 8 days in 'Pedro Sato' guavas at 30 ºC and 24 hours of wetness duration (Figure 3).

According to the Power Law Model, the incubation periods estimated for fruits stored at 10°C were 16 (6 hours) and 15 days (24 hours) for both pathogens in 'Kumagai' guavas. For 'Pedro Sato', the estimated incubation periods were 14 and 15 days with 6 hours wetness, and 11 and 10 days for *C. acutatum* and *C. gloeosporioides*, respectively, with 24 hours of wetness period (Figure 2).

The equation parameters of the Power Law model, adjusted to the data of the incubation and latent periods of anthracnose caused by *C. gloeosporioides* and *C. acutatum* are presented in Table 3.

In this study, the incubation periods estimated at 10ºC were 15 and 10 days for 'Kumagai' and 'Pedro Sato" guavas, respectively. Storage at low temperatures – soon after harvesting – delays the emergence and

Table 3. Coefficients of determination (R^2) and parameters (*a* and *b*) according to the Power Law model equation [$y=at^b$, where *y* is the incubation or latent period (days), *t* is the temperature (ºC) and *a* and *b* are the model parameters] adjusted to the incubation period and latent period of anthracnose (*Colletotrichum gloeosporioides* and *C. acutatum*) in 'Kumagai' and 'Pedro Sato' guava fruits.

Analyzed Variables	Wetness period	a	B	R^2
		'Kumagai' guavas		
		C. gloeosporioides		
Incubation period	6 hours	46,43	0,48	0,77
Incubation period	24 hours	90,19	0,76	0,65
Latency period	6 hours	171,58	0,89	0,85
Latency period	24 hours	43,56	0,45	0,70
		C. acutatum		
Incubation period	6 hours	81,45	0,70	0,70
Incubation period	24 hours	66,81	0,66	0,72
Latency period	6 hours	129,95	0,82	0,50
Latency period	24 hours	47,65	0,47	0,63
		'Pedro Sato' guavas		
		C. gloeosporioides		
Incubation period	6 hours	144,96	0,92	0,83
Incubation period	24 hours	28,14	0,43	0,57
Latency period	6 hours	98,7	0,75	0,87
Latency period	24 hours	30,39	0,37	0,63
		C. acutatum		
Incubation period	6 hours	71,00	0,71	0,76
Incubation period	24 hours	21,93	0,36	0,47
Latency period	6 hours	61,69	0,06	0,80
Latency period	24 hours	41,16	0,46	0,66

reduces the development of *C. gloeosporioides* and *C. acutatum*, which can increase the commercialization time and reduce fruit damages.

Although there was a quite extensive range favorable to the disease, wetness duration started to be favorable from 6 hours, limiting the disease occurrence. Considering that by means of pruning and irrigation guava fruits are harvested throughout the year, a warning system using agrochemicals only in periods favorable to the disease could be proposed. This tool could be used to predict the pathogen onset, allowing opportunities to minimize its transmission and reduce the number of fungicide applications.

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