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Gray mold in immature fig fruit: pathogenicity and growth temperature

Mofo cinzento em frutos imaturos de figo: patogenicidade e temperatura para crescimento

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– NOTE –

ABSTRACT

*Several diseases can be associated with figs but recently a fruit rot was observed in green fruit. The purpose of this study was to determine the pathogenicity of **Botrytis** sp., to quantify incubation period (IP) and latent period (LP), to verify the optimum temperature for mycelial growth, and to identify the different species of **Botrytis** sp. isolated from immature figs. **Botrytis** sp. isolated from figs proved to be pathogenic to immature fruit with and without wounding the fruit surface and ostiole. The IP period was 3 days on fruit with wounds and 5 days on fruit inoculated within the ostiole (without wound). The LP was 6 days in all treatments. The optimum temperature for mycelial growth was 18°C. Inferred from sequences of a segment comprising the ITS region of ribosomal DNA concluded that the isolates are **Botrytis cinerea**.*

Key words: ***Ficus carica***, ***Botrytis*** *sp., incubation period, latency period.*

RESUMO

*Várias doenças podem estar associadas com figos, mas recentemente uma podridão dos frutos foi observada em frutos verdes. O objetivo deste estudo foi determinar a patogenicidade de **Botrytis** sp., quantificar o período de incubação (PI) e o período de latência (PL), verificar a temperatura ótima para o crescimento micelial e identificar as diferentes espécies de **Botrytis** sp. isoladas a partir de frutos imaturos de figo. **Botrytis** sp. isolado a partir de figos provou ser patogênico em frutos imaturos com e sem ferimento na superfície dos frutos e no ostíolo. O PI foi de 3 dias em frutos com ferimento e 5 dias em frutos inoculados no ostíolo e sem ferimento. O PL foi de 6 dias em todos os tratamentos. A temperatura ótima de crescimento micelial foi18°C. A partir de sequências de um segmento que compreende a região de ITS do DNA ribossomal, concluiu-se que os isolados são **Botrytis cinerea**.*

Palavras-chave: ***Ficus carica***, ***Botrytis*** *sp., período de incubação, período de latência.*

The fig tree (***Ficus carica*** L.) is widely cultivated in the world due to its easy adaptation to different climates. The fruit is acceptable for consumption *in natura* (ripe), green (industry standard), and swollen or ramie. After harvesting, diseases can take place through direct access by natural openings (ostiole and peduncle) or mechanical damage (injuries) arising from handling (DURIGAN, 1999). During a survey carried out in the state of Paraná ***Botrytis cinerea*** was cited for causing symptoms in postharvest figs (VELLOZO et al., 2001). Throughout the world genus ***Botrytis*** that causes gray mold disease and was reported in several plants in both pre and post-harvest (TOFOLI et al., 2011). Occurrence of gray mold caused by ***Botrytis cinerea*** was also reported in postharvest figs (KWON et al., 2011), and in figs in pre and post-harvest in Korea (CHEONG et al., 2013). However, monocyclic components and the optimum temperature for the development of the pathogen have not been reported or investigated in Brazil, or in other parts of the world. Also, disease occurrence in immature fig fruit is still unknown.

The objectives of this study were: i) to prove the pathogenicity of ***Botrytis*** sp. to immature fig fruit; ii) to quantify the incubation period and the latent period of the pathogen on the immature fig fruit of the cultivar 'Roxo de Valinhos'; iii) to verify the minimum, maximum and optimum temperature for mycelial growth of the pathogen and; iv) to identify

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the specie of ***Botrytis*** sp. by means of sequences of ITS1 - 5.8 S - ITS2 Rdna. The ***Botrytis*** sp. mycelial growth and sclerotia signs were observed in immature fruits of the cultivar 'Roxo de Valinhos' harvested from domestic organic orchard, after one month of storage at 10°C (Figure 1A). Hyphae fragments of the pathogen associated with the immature fruit were transferred to a fresh Petri dish containing potato-dextrose agar (PDA). Isolates were incubated for 5 days at 22±2°C, with light regime of 12 hours dark and 12 hours light.

After incubation, the formation of grayish colonies appeared and the characteristic structures of ***Botrytis*** sp. were observed by optical microscopy (Figure 1B). Colonies were purified by single spore isolation using water agar (WA) media. One of the purified isolate, maintained on PDA at 22±2°C (Figure 1C), was named BCF01-LEMID and used in this study. For the pathogenicity test, 33 immature fruits of the cultivar 'Roxo de Valinhos' (***Ficus carica*** L.) were used. All fruits were organically produced by the Canguiri Experimental Farmin Pinhais, Paraná State. Fruits were disinfected with 70% ethanol (1min), 1% sodium hypochlorite (1 min), and washed three times with sterile distilled water. For inoculation, mycelial plugs of 5mm diameter isolated from a 7-days-old single spore culture were placed onto the ostioles opening, at the equatorial region of the immature fruit with and without wound (11 fruits per treatment). Wounds of about 2cm depth were done using a sterile needle. Fruits were incubated at 22±2°C and lesions diameter were measured every 24 hours for 6 days, by averaging two perpendicular diameters. After the onset of signs on the lesion (Figure 1D-E), the fungus was re-isolated in PDA media to confirm the Koch´s postulates. The experiment was repeated twice.

For the evaluation of mycelial growth, the 5mm diameter mycelium disks were collected from the 7 days old colonies edge and transferred into the center of Petri dishes (7cm diameter) containing PDA medium (Himedia®, Sao Paulo, Brazil). The plates were incubated at temperatures of 5, 10, 15, 20, 25, 30 and 35°C (1st experiment), and at temperatures of 22, 24 and 27°C (2nd experiment) under a 12 hour

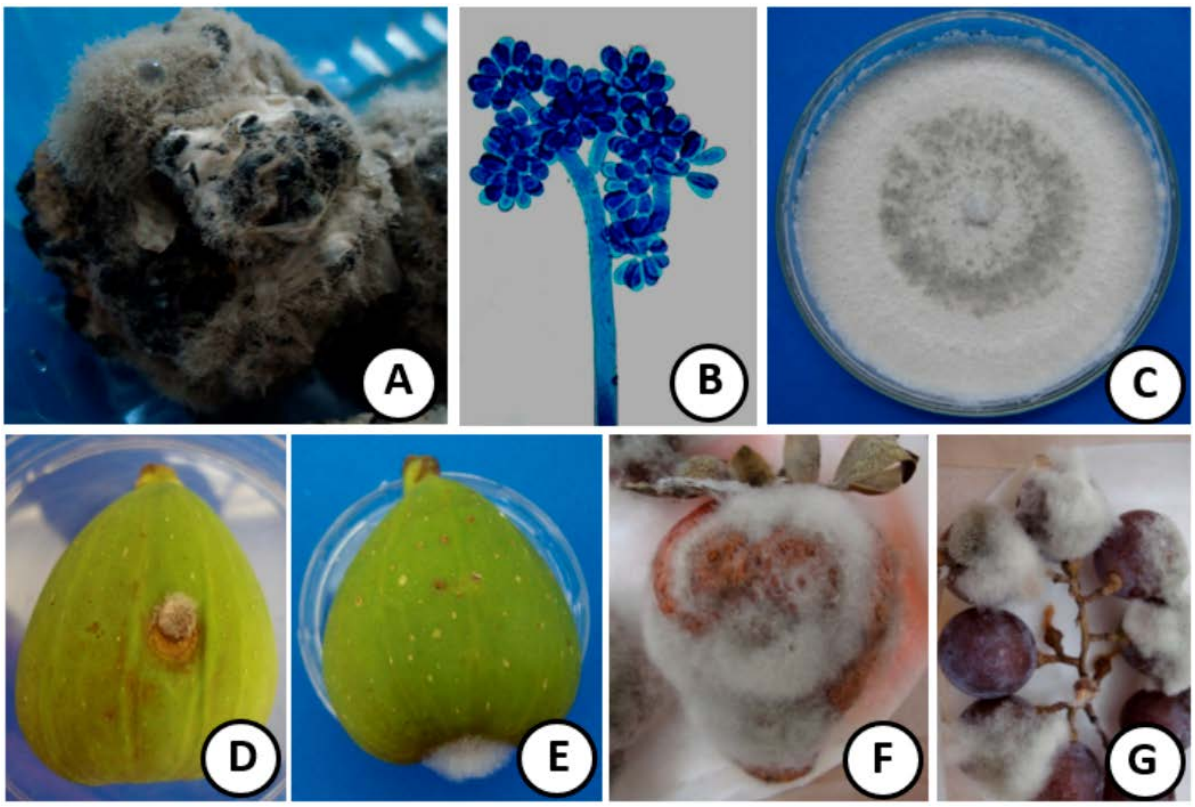

Figure 1 - Gray mold symptoms and signs of the causal agent ***Botrytis*** sp. Signs of ***Botrytis*** sp. (mycelium, conidia and sclerotia) found in immature fruit of fig stored in the refrigerator (A), light microscopy of conidia and conidiophores (B), colony of ***Botrytis*** sp. on PDA (Potato Dextrose Agar) (C), symptom induced by artificial inoculation in immature figs (***Ficus carica*** L.) of the cultivar 'Roxo de Valinhos' on wound in the equatorial region (D) and into the opening of the ostiole (E), symptom induced by artificial inoculation in strawberry (F), and symptom induced by artificial inoculation in grape (***Vitis labrusca***) of the cultivar 'Niagara' (G).

photoperiod. Daily measurements were made until the first colony of any treatment reach the edge of the plate. The experimental design was completely randomized with three replications used for each treatment. When determining the minimum, optimum and maximum temperatures for mycelial growth, the generalized Beta function was adjusted to the data collected in the latest evaluation, according to BASSANEZI et al. (1998).

The pathogenicity of ***Botrytis*** sp. isolated from fig was also tested in fruits of strawberry and grape by inoculating fungus PDA mycelium. Fruits were maintained under 25ºC and high humidity to confirm symptoms of the disease. For the molecular analysis fungal DNA was extracted, and the internal transcribed spacer (ITS) was amplified under the conditions described by WHITE JR. & MORROW (1990) with primers V9G (DE HOOG et al., 1998).

The pathogenicity of ***Botrytis*** sp. in immature figs were confirmed, demonstrating the ability of the pathogen to infect immature fruit inoculated with and without wound and also, through the opening of the ostioles. However, the wounded fruits obtained the greatest severity, with the development of mean diameter lesions of 4.10cm in the 1st experiment, and 4.13cm in the 2nd experiment, differing statistically from other treatments (Table 1). This result agrees with GARRIDO & SONEGO (2005), who reported the occurrence of symptoms caused by ***B. cinerea*** in grape berries when the material had lesions caused by pest insects, cracks or other injuries.

The immature fruit inoculated with ***Botrytis*** sp. onto the opening of the ostiole and without wound were not statistically different, and showed mean diameter lesions with of 3.46 cm and 3.52 cm respectively (Table 1). These results showed that the ostioles did not facilitate the pathogen penetration, contrary to FREIRE et al. (2006) which report that the penetration of several pathogens isolated from fig fruit with symptoms of decay was facilitated by the ostiole opening.

The incubation period of the pathogen inoculated in the wounded fruit was 3 and 2 days in the 1st and 2nd experiments, respectively. In fruit inoculated into the ostioles and without wound, the incubation period was 4 days. The latency period was 6 days for all treatments. There was an incidence of 100% in all treatments.

The development of ***Botrytis*** sp. isolated from fig fruit was significantly affected by temperature. The ideal temperature for d colonies development of ***Botrytis*** sp. isolated from fig fruit was 18°C (Figure 2). Minimum and maximum temperatures for the colonies development were 0 and 38°C, respectively. Similar result was observed by GARRIDO & SÔNEGO (2005), in which the development of ***Botrytis*** sp. was favored by temperatures ranging from 18 to 23°C.

Additionally, it is very important the establishment of disease management strategies for controlling the ***Botrytis*** sp. in pre- and post-harvest, mainly when temperature conditions are conducive for the disease development. It is also recommended to consider the inoculum amount in the area, and the possibility of inoculum sources from other orchards and cultures, as ***Botrytis*** sp. isolated from fig immature fruit was also able to infect and cause symptoms of gray mold in fruit of strawberry (Figure 1F) and grape (Figure 1G).

Amplification of the ITS rRNA region produced a fragment of 447bp, that showed 98.9-100% identity to that of the representative

Table 1 - Area under the disease progress curve (AUDPC) and mean diameter of lesions, six days after the inoculation of ***Botrytis*** sp. in immature fruit of fig (***Ficus carica*** L.) cultivar 'Roxo de Valinhos' with wound, without wound and into the opening of the ostiole. Curitiba-PR, 2012.

Treatment	-----Experiment 1-----		-----Experiment 2-----	
	AUDPC*	Maximum diameter*	AUDPC*	Maximum diameter*
With wound	9.6 a	4.1 a	11.0 a	4.1 a
Without wound	5.7 b	2.7 b	7.5 b	3.5 b
Ostiole	6.1 b	3.1 b	7.9 b	3.5 b
CV (%)	34.2	25.5	27.7	12.6

*Means followed by the same letters in the columns do not differ at 5% probability by the Tukey test.
CV= Coefficient of Variation.

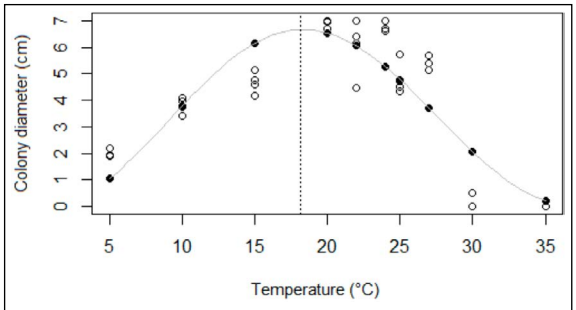

Figure 2 - Diameter of the colonies of ***Botrytis*** sp. maintained on PDA at different temperatures for 10 days, and adjustments on the generalized Beta distribution according to BASSANEZI et al. (1998).White circles represent the repetitions of each tested temperature; black circles were clear-cut by the statistical analysis used.

strains of ***B. cinerea*** sequences deposited in the Genbank (Accession No. KT630651).This is the first report of the occurrence of this pathogen in immature fig fruit in Brazil.

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