

Effect of temperature and photoperiod on the *in vitro* germination of conidia of *Botrytis squamosa*, the causal agent of Botrytis leaf blight of onion

Leandro Luiz Marcuzzo¹, Katiani Eli¹

¹Instituto Federal Catarinense - IFC / Rio do Sul Campus, CP 441, CEP 89163-356, Rio Sul, SC, e-mail: marcuzzo@ifc-riodosul.edu.br

Autor para correspondência: Leandro Luiz Marcuzzo (marcuzzo@ifc-riodosul.edu.br)

Data de chegada: 24/02/2016. Aceito para publicação em: 17/05/2016.

10.1590/0100-5405/2163

ABSTRACT

Marcuzzo, L.L.; Eli, K. Effect of temperature and photoperiod on the *in vitro* germination of conidia of *Botrytis squamosa*, the causal agent of Botrytis leaf blight of onion. *Summa Phytopathologica*, v.42, n.3, p.261-263, 2016.

Temperature and photoperiod have a direct effect on spore germination and, for *Botrytis squamosa*, there is scarce information on this subject. Learning the biology of the causal agent has a great importance to understand the development of the disease in the field, as well to take control measures. With the aim of evaluating the influence of temperature and photoperiod on *B. squamosa* conidial germination, an experiment was conducted in a completely randomized design with four replicates. A 0.1-ml suspension of 1.65×10^5 conidia per ml was added and spread on Petri dishes containing 1% water-agar. The Petri dishes were placed in BOD incubators adjusted to 10, 15, 20, 25 and 30°C without light, and the percentage of germination of 100 conidia was evaluated under an optical microscope by measuring the germ tube after 24 hours of incubation.

Keywords: *Allium cepa*, biology, environment.

In a second moment, this experiment was repeated by incubating the spores at 23°C and photoperiods of 0, 6, 12, 18 and 24 hours, and by evaluating the percentage of germination after 24 hours. Results showed that the highest germination percentages can be obtained in the temperature range between 10 (79.75%) and 30°C (92.5%), and the optimum temperature for germination is 23°C. In relation to photoperiod, the highest germination percentage (94%) was obtained without light, which indicates that *B. squamosa* germination is favored by shorter periods of light. Thus, the information obtained on the biology of the causal agent of botrytis leaf blight allows better understand the epidemiology of this disease, and such results can be used to help develop a disease forecasting system.

RESUMO

Marcuzzo, L.L.; Eli, K. Efeito da temperatura e do fotoperíodo na germinação *in vitro* de conídios de *Botrytis squamosa*, agente da queima das pontas da cebola. *Summa Phytopathologica*, v.42, n.3, p.261-263, 2016.

A temperatura e o fotoperíodo têm efeito direto na germinação de esporos e para *Botrytis squamosa* as informações são escassas sobre o assunto. O conhecimento da biologia do patógeno é de grande importância para compreender o desenvolvimento da doença no campo, bem como para tomar as medidas de controle. Com o objetivo de avaliar a influência da temperatura e do fotoperíodo sobre a germinação de conídios *B. squamosa*, um experimento foi conduzido em delineamento inteiramente casualizado com quatro repetições. Uma suspensão de 0,1 ml contendo $1,65 \times 10^5$ conídios por ml foi adicionado e espalhado em placas de Petri contendo Ágar-Água 1%. As placas foram depositadas em estufa ajustadas para 10, 15, 20, 25 e 30°C sem luz, sendo avaliada a percentagem de germinação de 100 conídios em microscópio óptico através da mensuração do tubo germinativo após 24 horas de incubação. Em

um segundo momento repetiu-se o experimento incubando os esporos a 23°C com os fotoperíodos de 0, 6, 12, 18 e 24 horas avaliando-se a percentagem de germinação após as 24 horas. Os resultados demonstraram que as maiores percentagens de germinação são obtidas no intervalo de temperatura entre 10 (79,75%) a 30°C (92,5%), tendo como a temperatura ideal para a germinação a temperatura de 23°C. Em relação ao fotoperíodo, obteve-se a maior percentagem (94%) de germinação quando esteve sem luz, assim é possível inferir que *B. squamosa* tem sua germinação favorecida por menores períodos de luz. Deste modo, as informações obtidas sobre a biologia do agente causal da queima das pontas, permitem um maior entendimento da epidemiologia da doença, e tais resultados podem ser utilizados para auxiliar na elaboração de um sistema de previsão da doença.

Palavras-chave: *Allium cepa*, biologia, ambiente.

Botrytis leaf blight of onion, caused by *Botrytis squamosa*, stands out as the most important disease affecting this crop in the seedling stage. This disease is widespread in temperate climate regions, where there are frequent periods of moderately warm temperatures, high relative humidity and low light intensity (7). Epidemics are favored by temperatures between 20-25°C, relative humidity above 75%, and extended periods of leaf wetness (4).

Symptoms of botrytis leaf blight are characterized by whitish lesions on the leaves, which are generally surrounded by a greenish-white halo. Initially, the lesions occur isolated and there is not sporulation on the green tissue. However, the most characteristic symptom of damage to

the plant is leaf burn, occurring from the apex to the base of the leaf, where there is sporulation of intense translucent appearance on the necrotic part of the leaf (6).

According to Wordell Filho & Boff (8), the dissemination of *B. squamosa* conidia occurs from volunteer infected plants, onion crops nearby, or chives. Conidial germination can occur between 6 and 33°C, with optimal range defined between 20 to 28°C. Infection can occur in temperature ranges of 6 to 28°C with optimum range between 15 to 20°C.

Learning the biology of *B. squamosa* is of great importance to understand the development of this disease under field conditions and

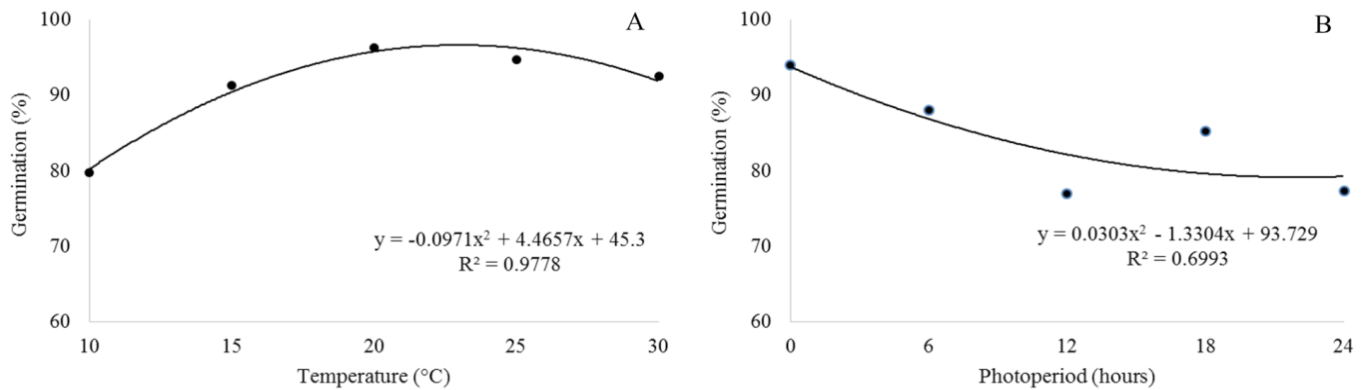


Figure 1. *B. squamosa* conidial germination curve at different temperatures (A) and photoperiods (B). IFC/Campus Rio do Sul, 2015.

find control alternatives. Thus, this study aimed to evaluate, under *in vitro* conditions, the influence of temperature and photoperiod on conidial germination of *B. squamosa*.

This study was conducted at the Laboratory of Microbiology and Plant Pathology at the “Instituto Federal Catarinense”, Campus Rio do Sul, and *B. squamosa* isolate was obtained from onion plants with symptoms of botrytis leaf blight; spores collected from the lesions on the leaves were multiplied in PDA medium. The experiment was conducted in a completely randomized design with four replicates.

The isolate was grown on Petri dishes containing PDA medium for seven days at 25°C and 12 hours of photoperiod. Thereafter, conidia were removed from the mycelial growth by washing with sterile water; then, 100 µl of conidial solution at a concentration of 1.65×10^5 conidia/mL, determined in a Neubauer chamber, were spread with a Drigalski spatula on Petri dishes containing agar-water (1%) medium. After inoculation, the Petri dishes were incubated in BOD chambers at temperatures of 5, 10, 15, 20, 25, 30 and 35 °C ($\pm 1^\circ\text{C}$) and photoperiod of zero hours. In a second moment, the experiment was repeated by incubating *B. squamosa* conidia in BOD at 23°C ($\pm 1^\circ\text{C}$) (optimum germination temperature found in the temperature experiment) and photoperiods of 0, 6, 12, 18 and 24 hours.

In both parts of the experiment, germination percentage was evaluated after 24 hours of incubation. To visualize the conidia, a few drops of 0.33% methylene blue were added to a Petri dish and spread in circular movements until the entire surface of the dish was covered. Conidial germination was determined by randomly counting 100 conidia under an optical microscope with 40X lens. Germinated conidia were considered those with a germ tube greater than the conidial size.

The obtained results indicated that the temperature has great influence on *B. squamosa* conidial germination. At temperatures between 10 and 30°C (Figure 1A), germination was high, 79.75 and 92.5%, respectively. The highest germination percentages occurred in the range of 15 to 30°C. Similar results were found by Alderman & Lacy (1), who established that *B. squamosa* conidial germination occurs in temperature ranges from 6 to 33°C, optimum temperature between 20 and 28°C. According to Swanton (5), *B. squamosa* conidial germination is abruptly reduced at temperatures above 33°C, confirming the data obtained in the present study, since at 35°C germination percentage was 1.75%, decreasing by 90.75% in relation to that at 30°C.

Conidial germination was also abruptly reduced to 3.75% at the temperature of 5°C. From 10°C to 5°C, germination percentage decreased by 76%. As average temperatures of 5 and 35°C are atypical conditions for onion growing in Brazil and since there was not curve

adjustment, conidial germination data were chosen from the range of 10 to 30°C, based on the equation generated by the curve (Figure 1A) in which the temperature of 23°C was optimal for conidial germination. Similar results were found in assays performed by McDonald (3), who evaluated conidial germination of *B. squamosa* inoculated in aqueous suspension, where the temperature of 24°C was considered optimum for germination.

In Figure 1B, the influence of photoperiod on *Botrytis squamosa* conidial germination can be observed, where the photoperiod ends up causing an inhibitory effect on germination; consequently, the higher percentage (94%) was obtained without light (Figure 1B). Similar results were found by Borges Neto et al. (2), who evaluated the influence of photoperiod on the development of *Fusarium graminearum* and observed that the greater development of the pathogen occurred in the dark. Thus, it is possible that *B. squamosa* has its germination favored by shorter periods of light; therefore, cloudy days of low light intensity, such as several days that occur in the fall/winter in the region of Alto Vale do Itajaí during the onion crop cycle, favor spore germination and the disease occurrence.

It was concluded that *Botrytis squamosa* conidial germination is influenced by temperature and photoperiod, since the highest germination percentage can be obtained at temperatures from 15 to 30°C, optimum temperature of 23°C, and zero hours of photoperiod. From the obtained results, it can be inferred that the development of botrytis leaf blight in the field in the region of Alto Vale do Itajaí, Santa Catarina State, Brazil, during the crop cycle is favored by mild temperatures and many cloudy days with low light intensity.

The data obtained for temperature and photoperiod on *B. squamosa* conidial germination help better understand the biology of the causal agent of botrytis leaf blight of onion and therefore the epidemiology of this disease in the field. These results will support the development of a disease forecasting system.

REFERENCES

1. Alderman, S.C.; Lacy, M.L. Influence of dew period and temperature on infection of leaves by dry conidia of *Botrytis squamosa*. **Phytopathology**, St. Paul, v.73, n.8. p.1020-1023, 1983.
2. Borges Neto, C.R.; Gorgati, C.Q.; Pitelli, R.A. Influência do fotoperíodo e da temperatura na intensidade de doença causada por *Fusarium graminearum* em *Egeria densa* e *E. najas*. **Fitopatologia Brasileira**, Brasília, v.29, n.3, p. 252-258, 2004.
3. McDonald, M.R. **Effect of environmental and host factors on Botrytis leaf blight of onion**. 1981. 108f. Thesis, University of Guelph, Guelph.

4. Nunes, M.E.T.; Kimati, H. Doenças do alho e cebola. In: Kimati, H.; Amorim, L.; Bergamim Filho, A.; Camargo, L.E.A.; Rezende, J.A.M. (Ed.). **Manual de Fitopatologia**. 4. ed. São Paulo: Ceres, 1997. V.2, cap.7, p.55-69.
5. Swanton, C.J. **Influence of environmental factors on development and control of *Botrytis* leaf blight of onions**. 1977. 89f. Thesis, University of Guelph, Guelph.
6. Tofoli, J.G.; Ferrari, J.T.; Domingues, R.J.; Nogueira, E.M.C. *Botrytis* sp. em espécies hortícolas: hospedeiros, sintomas e manejo. **Biológico**, São Paulo, v.73, n.1, p.11-20, 2011.
7. Wordell Filho, J.A.; Boff, P. Queima acizentada. In: Wordell Filho, J. A.; Rowe, E.; Gonçalves, P.A.; Debarba, J.F.; Boff, P.; Thomazelli, L.F. **Manejo Fitossanitário na cultura da cebola**. Florianópolis: EPAGRI, p.19-30, 2006.
8. Wordell Filho, J.A.; Stadnik, M.J. Controle da mancha acizentada da cebola e seu impacto sobre a qualidade de mudas. **Horticultura Brasileira**, Brasília, v.24, n.4, p.437-441, 2006.