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The combined effects of temperature and leaf wetness periods on soybean frogeye leaf spot intensity

Interação entre temperatura e período de molhamento foliar na intensidade da mancha foliar olho-de-rã

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Abstract

The frogeye leaf spot, a disease caused by the fungus *Cercospora sojina*, affects soybean crops worldwide with enormous economic impact. In this study, we evaluated the combined effects of temperature and duration of leaf wetness periods on the intensity of frogeye leaf spot in soybean. Experiments were conducted in a growth chamber with cultivar Don Mario 7.0i at temperatures of 15, 20, 25, 30 and 35°C and leaf wetness periods of 12, 24, 36, 48 and 72 hours. The experimental design was completely randomized with five replications. When soybean plants were grown at 15°C, affected leaflet area, number of lesions per leaflet and diameter of lesions could only be measured after 60 hours of leaf wetness. At the temperatures of 20 and 25°C this period was reduced to 24 hours of leaf wetness, at 30ºC, we found the need for 36 hours of leaf wetness and at a temperature of 35°C, 48 hours. The optimal temperatures for disease development were 27°C for diameter and affected leaflet area and 28°C for number of lesions per leaflet with 72 hours of leaf wetness.
Key words: *Cercospora sojina*, *Glycine max* L.

Resumo

A cultura da soja apresenta grande importância mundialmente, entre as doenças que ocorrem nesta cultura está à mancha olho-de-rã (causada por *Cercospora sojina*). O objetivo deste trabalho foi avaliar a interação entre temperaturas e diferentes períodos de molhamento foliar na intensidade da mancha olho-de-rã em soja. O experimento foi conduzido em câmara de crescimento com a cultivar Don Mario 7.0i nas temperaturas de 15, 20, 25, 30 e 35ºC e os períodos de molhamento foliar de 12, 24, 36, 48 e 72 horas. Cinco experimentos foram realizados, um para cada temperatura, l5, 20, 25, 30 e 35ºC e os períodos de molhamento foliar 12, 24, 36, 48, 60 e 72 horas consistiram os tratamentos, o delineamento experimental foi inteiramente casualizado com cinco repetições. Quando as plantas foram submetidas à temperatura de 15ºC, severidade foliar, número de lesões por folíolo e diâmetro da lesão foram observados somente com 60 horas de molhamento foliar, para temperaturas de 20 e 25ºC com 24 horas de molhamento foliar, para temperatura de 30ºC com 36 horas de molhamento foliar e para temperatura de 35ºC com 48 horas de molhamento foliar. A temperatura ótima estimada para o desenvolvimento da doença foi de 27ºC para severidade e diâmetro de lesões e de 28ºC para número de lesões por folíolo, com 72 horas de molhamento foliar.
Palavras-chave: *Cercospora sojina*, *Glycine max* L.

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Introduction

Numerous diseases affect soybean crops [*Glycine max* (L.) Merr.], among which, the frogeye leaf spot, caused by the fungus *Cercospora sojina* Hara, 1915, represents one of the most economically harmful. In the United States alone, estimated annual losses ranged from 183,868 to 345,148 metric tons between 2006 and 2009 (KOENNING; WRATHER, 2010).

In Brazil, a contaminated seed sample of the Bragg cultivar originated in the United States introduced the fungus in the state of Parana during the 1970/71 crop. No official data exists on the impact of frogeye leaf spot to soybean production since then. Nevertheless, frogeye leaf spot was the first disease to reach epidemic proportions in Southern Brazil (YORINORI; KLINGELFUSS, 2000), an event that marked the beginning of the soybean breeding program focused on genetic resistance to disease. Today, the use of resistant cultivars is the most efficient and cost effective means of controlling frogeye leaf spot, and is the main disease control measure adopted in Brazil (MIAN et al., 2009). This pathogen has not been associated with disease in any other crop or weed host (MIAN et al., 2008).

Disease development depends on the interaction among a susceptible plant, a virulent pathogen and a favorable environment. Host susceptibility and pathogen virulence remain relatively stable in the short term, whereas the environment may undergo frequent and important alterations even during a crop cycle. Environmental conditions mostly affect the onset of infection, potentially preventing it even when the host is susceptible and pathogens are present (BEDENDO; AMORIM, 2011). The presence of liquid water on leaf surfaces represents an important factor for the infectious process. The leaf wetness period refers to the period of time during which plant leaves remain moist (SUTTON et al., 1984). Dew water is essential for leaf wetness, especially during drier seasons. In parallel, temperature works as a catalyst agent for biological processes, thus, plants and pathogens require a minimal temperature to grow and maintain normal activity levels. In general, pathogens become more active in higher temperatures, when conditions are favorable, they more easily infect plants and cause disease (REIS; BRESOLIN, 2004). In summary, the infectious process of fungal diseases heavily depends on the interaction of leaf wetness periods and temperature.

In vitro studies indicated that the optimal temperature for conidia germination under continuous light was 22.4°C (CAMERA et al., 2013), whereas other work showed that mycelia growth and sporulation was optimized at 25°C, with slower growth occurring at 32°C (CRUZ, 2008). Veiga (1973) reported that 48 h of wetness favors the onset of frogeye leaf spot. Data on disease progression under controlled and natural conditions have provided a critical tool for modeling epidemiological studies (DEL PONTE et al., 2006). Detailed knowledge of the conditions that favor infection and colonization allow for the development of management strategies that reduce pathogen establishment. Nevertheless, few studies have focused on *in vivo* effects of temperature and leaf wetness on the development of *C. sojina*. Therefore, the objective of this work was to determine the effects of temperature and duration of continuous periods of leaf wetness on frogeye leaf spot.

Material and Methods

Experiments were carried out in Conviron growth chambers model E7 in a completely randomized design. Five experiments were carried out, one for each temperature tested, l5, 20, 25, 30 and 35ºC and, within each experiment, leaf wetness periods of 12, 24, 36, 48, 60 and 72 hours were tested. Each experiment was replicated five times.

We used isolate 25 of *C. sojina* provided by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Soja). This isolate came from soybean cultivar 'Cariri' from Balsas/MA, and fungal strain identification was performed through

differential cultivar set reactions (YORINORI; KLINGELFUSS, 2000). We first conducted a monosporic isolation (ALFENAS; MAFIA, 2007) and later multiplied the pathogen on tomato extract-agar (HINE; ARAGAKI, 1963). We used an inoculum concentration of 40 x 10^3 conidia mL^{-1} measured with a Neubauer hematocytometer (ALFENAS; MAFIA, 2007).

Four plants of Don Mario 7.0i cultivar were sown in 2L volume pots, and placed in a growth chamber at 25ºC temperature and 12-hour photoperiod. Plants were inoculated when the second trifoliate leafs were totally developed by spraying the spore suspension to run-off. As an untreated control, five pots were sprayed with water and kept in a moist chamber that maintained continuous leaf wetness at different temperatures. At the end of each period, plants were dried with a fan and maintained in their respective temperature.

Disease components evaluated included affected leaflet area as a percentage of total leaflet area (DISTÉFANO et al., 2009); leaflet lesion number, which is the number of frogeye lesions on each leaflet; and leaflet lesion diameter; the average diameter of four lesions per leaflet measured with a digital caliper, evaluated fifteen days after inoculation. We evaluated the last fully expanded trefoil of each plant.

The mean values of affected leaflet area, diameter, and number of lesions per leaflet and temperature data were submitted to regression analysis. Temperature and leaf wetness data fit the quadratic polynomial curve $Y= B1 X^2 – B2 X + B3$, where Y = the dependent variable (affected leaflet area, diameter, and number of lesions per leaflet), X = independent variable (temperature), B1 = estimated asymptote maximum B2 = parameter related to the initial inoculum and B3 = rate of disease progression. To describe the combined effect of temperature and leaf wetness duration on affected leaflet area, lesion number and lesion diameter, a function was fitted to the average of each variable by nonlinear regression using the STATISTICA software (StatSoft, Inc., 2001).

Results and Discussion

Both temperature and the duration of leaf wetness affected frogeye leaf spot intensity. Inoculated soybean plants kept at l5ºC displayed affected leaflet area within 60 h of leaf wetness, whereas plants kept at 20 and 25ºC were affected within 24 h, those kept at 30ºC within 36 h, and the ones kept at 35ºC within 48 h. There were no disease symptoms within 12 h of leaf wetness regardless of temperature. The temperatures that maximized each variable were 27ºC, 28ºC and 27ºC for affected leaflet area, number and diameter of lesions, respectively. Mean temperature for the maximization of these variables was 27.3ºC (Figure 1). When correlating leaf wetness duration, lesion area, number and diameter, we observed that disease intensity increased as the duration of leaf wetness increased from 12 h, when no disease was detected, to a 72-h maximum, following a quadratic polynomial model (Figure 2).

Combining the monomolecular-beta generalized function equations [Z=0.00731*(T15)**1.472*(36T)**0.5051)] * [1.298/1+6185.84*EXP(0.1379*HW)] where T = temperature, HW = hours of leaf wetness, for the temperature and leaf wetness duration, we generated surface response graphs for affected leaflet area (Figure 3A), and lesion number per leaflet (Figure 3B). The graph for lesion diameter (Figure 3C) was generated with the equation [Z=0.00731*(T-15)**1.472*(36-T)**0.5051)]*(1.298*EXP(6125.84*HW)]. We concluded that, regardless of the variable analyzed, disease intensity was highest at 27ºC and with 70 h of leaf wetness.

Each pathogen-host system has its own critical weather period, few hours during which environmental conditions are favorable to infection, i.e. the infection site is continuously wet in a given temperature, so that spores germinate and parasites enter and become established in the host (REIS; WORDELL FILHO, 2004).

Figure 1. Quadratic polynomial model of frogeye leaf spot variables versus temperature for each level of leaf wetness. (A) affected leaflet area (%), (B) number of lesions per leaflet and (C) diameter of lesions per leaflet (mm) of frogeye leaf spot in soybean cultivar Mario 7.0i.

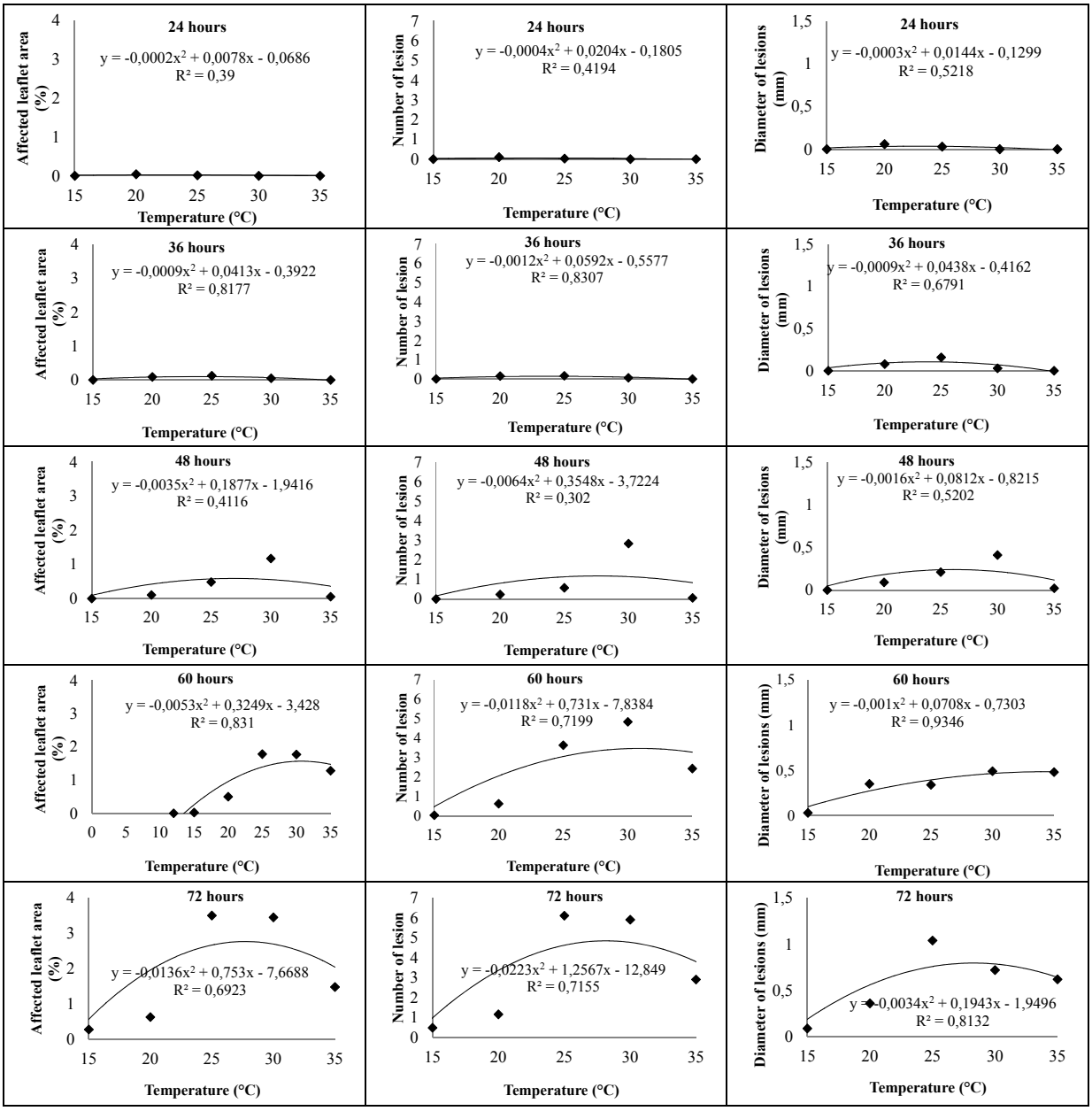
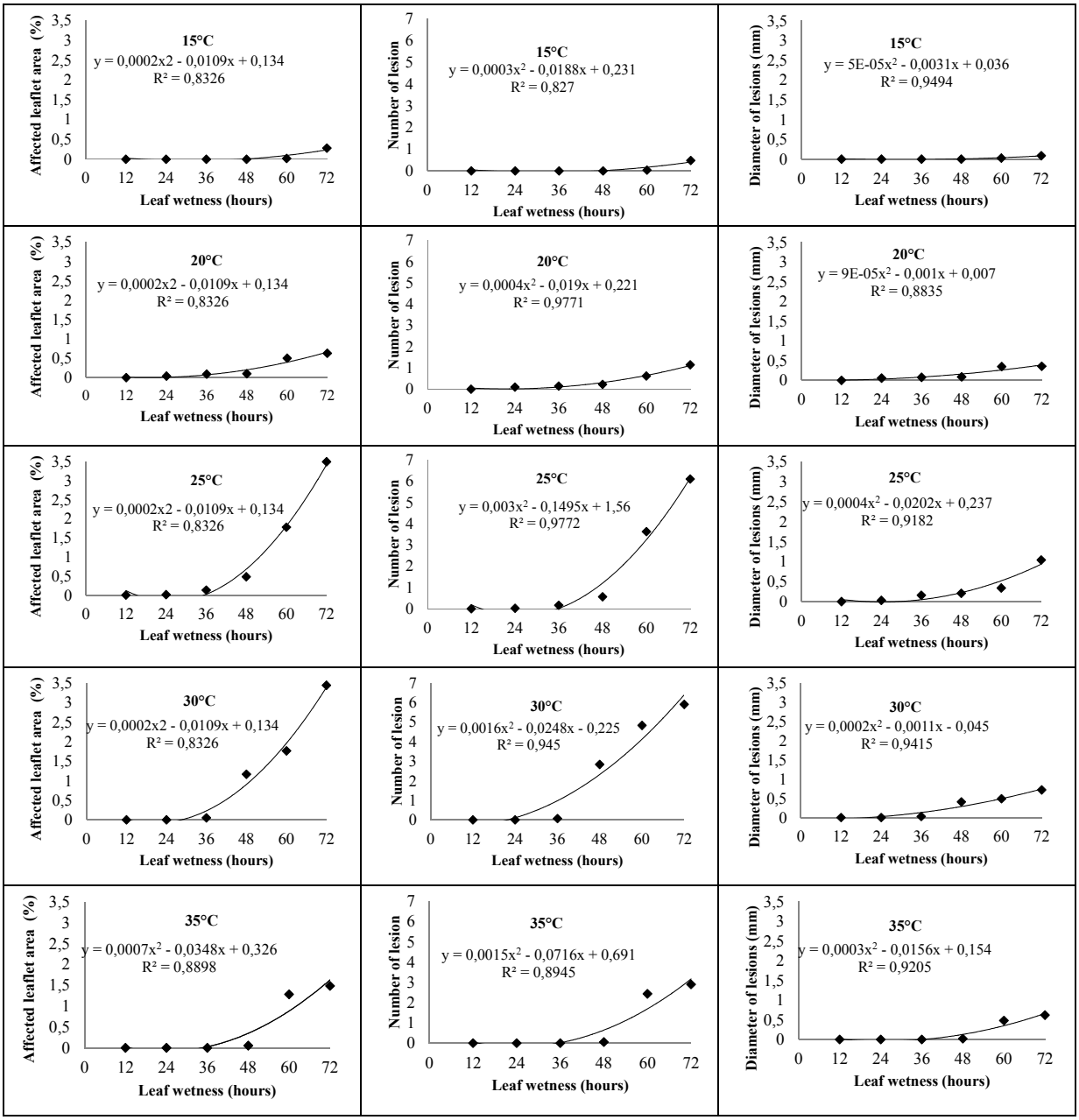


Figure 2. Quadratic polynomial model of frogeye leaf spot variables versus leaf wetness for each temperature level. (A) affected leaflet area (%), (B) number of lesions per leaflet and (C) diameter of lesions per leaflet (mm) of frogeye leaf spot in soybean cultivar Mario 7.0i.

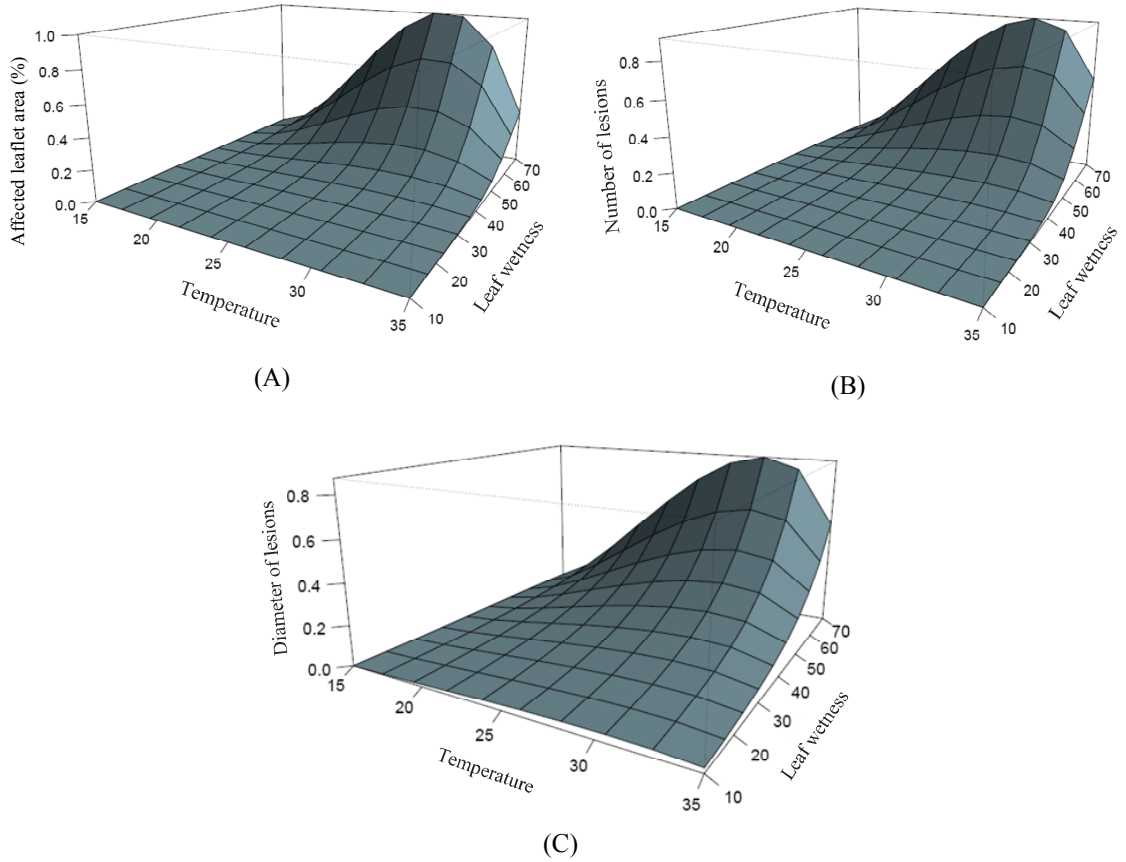


Here, we show that temperature and the duration of leaf wetness affect the intensity of frogeye leaf spot caused by *C. sojina*, with an optimum temperature for infection of 27.3ºC. Similarly, previous work indicated that *C. sojina* mycelial growth was maximized at 25ºC, and limited at temperatures higher than 32ºC. Infection and symptom development are favored by warm (25-30°C) and humid (>90% relative humidity) conditions, where sporulation can occur 48 h after the first symptoms become visible. Conidia can germinate on a leaf surface within an hour of deposition in the presence of water at 25 to 30°C with visible lesions developing 8 to 12 days after inoculation (PHILLIPS, 1999).

According to Alves et al. (2007), temperatures above 30º and below 15ºC affect the latent and infectious incubation periods of *Phakopsora pachyrhizi* Syd. & P. Syd., which often infect soybeans with *C. sojina*. These findings agree with the temperature data reported here for *C. sojina*.

Regarding leaf wetness duration, Veiga (1973) found that a 48-h wet period can result in infection, much as we observed, although we also found that the highest disease intensity occurs within a 72-h period. Kudo et al. (2011), showed that a 72-h wet period after *C. sojina* inoculation was sufficient to generate enough disease intensity for soybean genotype screening, a result that closely resembled those by Scandiani et al. (2010). Under these conditions, we observed the first symptoms on the eighth day after inoculation.

Figure 3. The effect of period of leaf wetness and temperature on (A) affected leaflet area (%), (B) number of lesions per leaflet and (C) diameter of lesions per leaflet (mm) of frogeye leaf spot in soybean cultivar Mario 7.0i.



Available data have allowed for the development of warning systems for bean angular leaf spot and anthracnose, caused by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun and *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara (REIS; BLUM, 2012). The model uses as input environmental conditions for the infectious process such as continuous leaf wetness duration and mean air temperature during this stage of the pathogen-host cycle. A warning system for *C. sojina* may also be developed from studies such as ours.

Conclusion

A temperature of 27°C and 72 hours of leaf wetness provided the ideal conditions for the infection and development of frogeye leaf spot in soybean plants.

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