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CROP PROTECTION

Number of lesions, severity and incubation period of isolates of *Corynespora cassiicola* in soybean cultivars

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ABSTRACT: This study checked the severity and incubation period of isolates of *Corynepora cassiicola* (C.c) in soybean cultivars, as well verified whether the position of leaflets influences the susceptibility of the plant to target spot. The experiment was conducted in randomized block with 8 isolates of C.c and 8 soybean cultivars factorial scheme, with five repetitions each block. The plants were inoculated with suspension from $2x10^4$ conidia mL^{-1}. The number of lesions per leaf and the incubation period (IP) were evaluated. The ISO 4S isolate caused greater number of lesions in plants. BMX Potência RR, BMX Força RR, and NA 5909 RG had the lowest occurrence of lesions, while the cultivar BMX Elite IPRO showed the highest number of lesions. More lesions were detected in the lower leaf compared to the upper leaf of the plants. The longest IP was observed for ISO 4S in both leaflets, while the lowest IP for ISO 1A, 3A and 2A in the lower leaf, and for ISO 11S and 1S in the median leaf. For cultivars, significant difference in IP was observed only in the lower leaf, with the longest and shortest IP detected for BMX Elite IPRO and BRS 284, respectively. In general, the upper leaf of the plants presented the shortest IP while the lower leaf had the longest IP.
Key words: *Glycine max*, epidemiology, target spot, phenological stage leaflets.

Número de lesões, severidade e período de incubação de isolados de *Corynespora cassiicola* em cultivares de soja

RESUMO: Este estudo teve como objetivo determinar o número de lesões e período de incubação de isolados de *Corynespora cassiicola* (C.c) em cultivares de soja, bem como verificar a influência da posição dos trifólios quanto a suscetibilidade a mancha alvo. O delineamento experimental foi de bloco causalizado em arranjo fatorial, oito isolados de C.c e oito cultivares de soja, com cinco repetições em cada bloco. As plantas foram inoculadas com suspensão de $2x10^4$ conídio mL^{-1}. Foram avaliados o número de lesões por folha e o período de incubação (IP). O isolado ISO 4S proporcionou maior número de lesões nas plantas. BMX Potência RR, BMX Força RR e NA 5909 RG apresentaram a menor ocorrência de lesões, enquanto a cultivar BMX Elite IPRO apresentou o maior número de lesões. Maior quantidade de lesões foram detectadas no trifólio inferior em comparação com o trifólio superior das plantas. O IP mais longo foi observado para ISO 4S em ambos os trifólios, enquanto o IP mais curto para ISO 1A, 3A e 2A no trifólio inferior, e para ISO 11S e 1S, trifólio mediano. Para as cultivares, observou-se diferença significativa no IP apenas no trifólio inferior, sendo o maior e menor IP constatados para BMX Elite IPRO e BRS 284, respectivamente. De modo geral, o trifólio superior das plantas demostrou o menor IP, enquanto o trifólio inferior o maior IP.
Palavras-chave: *Glycine Max*, epidemiologia, mancha alvo, estágio fenológico folíolos.

INTRODUCTION

Target spot disease, caused by the fungus *Corynespora cassiicola* (Berk. & MA Curtis) "C.T." Wei, was reported in soybean cultivated (*Glycine max* [L.] Merril) in the Brazilian territory for the first time in the state of Paraná, and later in the state of São Paulo (ALMEIDA et al. 1976). Previously considered a disease of minor importance in soybean cultivation, target spot has resurfaced as an endemic disease in recent decades due to the increase in monoculture, the use of susceptible cultivars, and decrease in pathogen sensitivity to fungicides (GODOY et al. 2021; XAVIER et al. 2013). GODOY et al. (2021), reported a reduction in soybean yield of up to 35 % in cultivars susceptible to target spot, while mentioned losses of up to 40 % in productivity.

Target spot leaf symptoms begin as small brown punctuations with yellow halos, progressing to large circular spots, light brown to dark, with concentric rings of up to 2 cm in diameter (ALMEIDA et al. 2005; SNOW et al. 1989). Spots may appear in greater numbers, but they are small, with 1 to 3 mm in diameter (GODOY et al. 2016). According to ALMEIDA et al., 2005, susceptible soybean cultivars may suffer severe defoliation, with spots on the stem and pods. The infection occurs at temperatures of 20 to 30 ºC, with a relative humidity of 80%. Under

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field conditions, the disease symptoms are observed at the beginning of the reproductive stages, when the canopy closes (ALMEIDA et al. 2005; TERAMOTO et al., 2013). Stated that target spot severity can affect up to 37% of the lower stratum of the plant without reducing the production of a susceptible cultivar, due to the low contribution of this stratum to form and fill seeds. However, the disease reduces plant yield when it occurs in the middle stratum of the plant since this region has great light interception and greater contribution in the formation and filling of seeds (SAKAMOTO et al. 1967).

The use of chemical products is the main form of controlling leaf diseases (BOLLER et al. 2007). The fungicides for end-of-cycle disease complex (ECD) are the same ones recommended for the control of target spot in the aerial part of the soybean crop, as follows: azoxystrobin + prothioconazole + mancozeb, difenoconazole+ prothioconazole + man-cozeb, prothioconazole + fluindapir + mancozeb and prothioconazole + mancozeb, fol-lowed by prothioconazole + fluxapyroxad and bixafen + prothioconazole + trifloxystrobin (GODOY et al., 2021). In many cases, it is the only efficient and economically viable measure to guarantee a high yield.

However, excessive use or inadequate doses of pesticides can lead, in the long term, the selection of phytopathogens resistant to the chemical substances used (HAWKINS et al. 2019). Addition, the excessive application of chemicals in crops and the indiscriminate use of fungicides is damaging to the environment and escalates production costs, polluting water-courses and directly affecting in pollinator populations (LUCHINI et al. 2000; ZHANG et al. 2018). As well as, *C. cassiicola* presents a high risk of becoming resistant to fungicides (FRAC, 2019; FRAC, 2020), causes negative impacts on the environment, as well as leaving undesirable residues in food, either by drift or by not adhering to days to harvest interval (MOREIRA et al. 2002; SRIVASTAV, 2020).

Including alternative forms of control in the integrated management of diseases is desirable. For instance, the adoption of strategies such as the use of cultivars that are more tolerant to pathogens can be an economically viable alternative, causing less environmental impact (HENNING et al. 2005). Evaluating disease progress in plants is essential to estimate the importance of a disease in different geographic regions (THEODORO et al. 2006). Parameters related to a host's response to infection by a pathogen, severity, and incubation period can interfere with epidemic progress (BERGAMIN FILHO, 1976).

Understanding the factors that influence the progress of the disease in the soybean ecosystem - *C. cassiicola* pathosystem is essential to identify conditions that influence epidemic development. Such knowledge would also contribute to the development of efficient management measures, since different isolates from one host can present differences on their virulence on other hosts, for example, cotton culture.

Despite the importance of the target spot for the state of Paraná, little research has been directed to identify the characteristics of the pathogen in different regions. Therefore, the objective of this study was to investigate the variability between *C. cassiicola* isolated obtained from cotton and soybean from different regions of Brazil, to determine the disease severity and incubation period in soybean cultivars, as well as to verify the position of the plant leaflets it is more susceptible to infection of the target spot.

MATERIALS AND METHODS

Soybean cultivars and growth conditions

The commercial soybean cultivars used in this study were: BMX Elite IPRO (GMO - Genetically Modified Organisms), BMX Força RR (GMO), BMX Garra IPRO (GMO), BMX Ponta IPRO (GMO), BMX Potência RR (GMO), BRS 284 (conventional), NA 5909 RG (GMO), and SYN 1562 IPRO (GMO). These cultivars were selected because they present some level of susceptibility to target spot.

Two seeds of each soybean cultivar were distributed in the plastic pots, after ten days the plants were thinned, leaving one plant per pot. In each plastic pot (1 liter) it contained sifted soil (distroferric red latosol) and five g of granulated chemical fertilizer of formulation 4-30-10 (N-P_2O_5-K_2O). The pots were kept in a greenhouse until the time of inoculation with *C. cassiicola* isolates (25 days).

Corynespora cassiicola isolates

Eight isolates of *C. cassiicola* deposited in the collection of the Seed Pathology Laboratory of the Rural Development Institute (IDR-Paraná) were used. The isolates were obtained from lesions of cotton, being ISO 1 A (Sertanópolis - PR), ISO 2 A (Jataizinho - PR), and ISO 3 A (Porecatu - PR) and soybean plants ISO 1 S (Goiânia - GO), ISO 2 S (Arapongas - PR), ISO 3 S (Sorriso - MT), ISO 4 S (Londrina - PR), and ISO 11 S (Diamantino - MT) collected in the field in the 2018/19 season. All isolates were identified and characterized, data published by PUIA et al., 2021, using the key of the genus *C. cassiicola* proposed by ELLIS (1957).

The isolates were purified by monosporic cultivation and stored at 4 °C using the Castellani method (FIGUEIREDO et al. 1967). Each *C. cassiicola* isolate was multiplied in 50 Petri dishes (diameter = 9 cm) containing V8 Juice® agar culture medium (V8: 5 g L^{-1} carbonate, 340 mL V8 juice, 34 g L^{-1} of agar) with streptomycin sulfate (500 mg L^{-1}). The plates were maintained at 25 ± 2 ºC and a 12/12 light-dark photoperiod for 10 days. After this period, the colonies of *C. cassiicola* showed abundant sporulation and were used as inoculum.

Experimental design

The experiment was performed using the randomized block design (RBD) in an 8 x 8 factorial scheme (eight *C. cassiicola* isolates x eight soybean cultivars), with five repetitions for each block over time. The blocks were formed over time, with use of the same type of sieved soil (distroferric red latosol) in the vessels, there are no variations in weather conditions between the blocks installed over temperature (26 ± 1 ºC) and moisture (80 ± 10%). The experimental unit of each block consisted of two pots containing one plant each.

Inoculation conditions

When soybean cultivars presented phenological stage V4, characterized by the third fully developed trifoliate leaf (MELO, 2009), were inoculated with *C. cassiicola* isolates. Under greenhouse conditions (temperature of 29 ± 1 ºC, relative humidity of 60 to 70%), the plants received 1 g of urea (CH_4N_6O) per pot, to stimulate the opening of plant stomata, 24 hours before inoculation.

For each isolate of *C. cassiicola*, a suspension of conidia was prepared at concentration of 2 x 10^4 conidia mL^{-1}, in which one drop per liter of the surfactant Tween 20 and 0.4% of unflavored gelatin were added. These adjuvants were added to improve the distribution of the inoculum suspension and the adhesion on soybean leaflets during inoculation. Because the greenhouse conditions did not favor infection by the pathogen, the pots with the plants were transferred to a climatized chamber with a temperature of 26 ± 1 ºC, relative humidity of 85 to 95%, and a photoperiod of 12/12 (light /dark), remaining in these conditions until the end of the evaluations at 20 days after inoculation (DAI).

The conidia suspensions were inoculated during the coolest period of the day (ap-proximately six o'clock the afternoon) on soybean leaves until the point of run-off, using a manual sprinkler.

Evaluations

The evaluations were carried out in the three leaflets of the 1st, 2nd, and 3rd trefoil, totaling nine leaflets per plant. All leaflets evaluated were fully expanded at the time of inoculation. The 1st, 2nd, and 3rd leaflets are located in the lower, middle, and upper parts of soybean plants in stage V4, respectively. For each isolate vs. cultivar combination, severity (number of lesions in 4 cm^2 of 1 leaflets) and the incubation period (days) were evaluated. The number of leaf lesions was determined by counting the lesions present within an area of 4 cm^2, delimited by a small frame (2 x 2 cm^2) using a permanent hydrographic ink pen (0.4 mm) in all leaflets.

The lesions were quantified daily, from the third DAI, over a period of 12 days. For the determination of the incubation period, we recorded the time elapsed from the inoculation to the moment the appearance of lesions in the leaflets ceased, stabilization of number of lesions (100%). From this information, the incubation period (IP) was considered as the period of time between inoculation and the appearance of 50% of symptoms (MERSHA et al. 2014), that is, the time for severity to equal K/2 + 1. Where IP is the incubation period expressed in days.

The number of lesions in 4 cm^2 and the incubation period were recorded for each leaf separately. The plant as a whole was also considered using the average values of the 1st, 2nd, and 3rd leaflets.

Statistical analysis

Data on the number of lesions per cm^2 in each leaf and incubation period were analyzed using the Aligned Rank Transform (ART), used for nonparametric factorial analyses by ANOVA (WOBBROCK et al. 2011). The aligned rank transformation allows non-parametric testing for interactions and main effects using standard ANOVA techniques. For the transformation of the original data the ARTool package (KAY et al. 2016). When significant differences were found, the means were compared using the Tukey test ($P < 0.05$) using the emmeans package (LENTH et al. 2018). Statistical tests were performed by the software R 4.0.2 (R CORE TEAM, 2020).

RESULTS

Severity – number of lesions

In all cultivars, *C. cassiicola* caused the greatest number of lesions in the lower part of the plants (1st leaf), which presented from 14.21 to 17.56

lesions per 4 cm2. The number of lesions in the middle part of the plants (2nd leaf), was from 10.50 to 13.70 lesions in 4 cm^2. The lowest occurrence of lesions, 7.10 to 8.00 lesions per 4 cm^2, was observed in the upper part of the plants. For the three evaluated leaflets, the highest occurrence of lesions was consistently detected in the cultivar BMX Elite IPRO, while on the 1st and 2nd leaflets, the cultivar BMX Potência RR presented the least number of lesions.

No significant interaction between isolate and cultivar was detected for numbers of lesions for the 1st, 2nd, and 3rd leaflets and whole plant average ($P = 0.70, 0.92, 0.86, 0.80$, respectively). On the other hand, a significant difference ($P < 0.01$) was observed for the number of lesions caused by the different isolates within each leaflet (1st, 2nd, and 3rd) and for the mean (Figure 1).

The highest number of lesions on the 1st leaf was caused by the ISO 4S isolate, with 18.02 lesions per 4 cm^2, differing from ISO 2A that resulted in a lower number of lesions, with 14.33 lesions in 4 cm^2. Remaining isolates produced intermediates numbers of lesions from 15.06 to 16.20 lesions per 4 cm^2.

On the 2nd leaf, the isolates ISO 4S, ISO 1A, and ISO 3S produced the highest number of lesions, with 13.04, 12.98, and 12.81 lesions per 4 cm^2, respectively, differing from ISO 2A that caused 10.06 lesions in 4 cm^2, the least value found.

For the 3rd leaf, the isolate that induced the highest number of lesions (9.15 per 4 cm^2) was ISO 4S, differing from the isolates ISO 2A, ISO 3A, ISO 2S, ISO 1S, ISO 3S, and ISO 11S, which resulted in fewer lesions, from 6.77 to 7.54 lesions in 4 cm^2.

When analyzing the average number of lesions on the whole plant, it was reported that the ISO 4S isolate caused the greatest number of lesions, 13.4 lesions per 4 cm^2, reflecting greater number of lesions on the plants.

Conversely, fewer lesions were caused by the isolates ISO 2A, ISO 2S, and ISO 11S, ranging from 10.39 to 11.66 lesions in 4 cm^2. The other isolates caused intermediate target spot severity (Figure 1).

In general, the different isolates of *C. cassiicola* caused the greatest number of lesions on the lower part of the plants (1st leaf), varying from 14.33 to 18.02 lesions in 4 cm^2 of leaf. For the middle part of the plants (2nd leaf), the value ranged from 10.06 to 13.04 lesions in 4 cm^2.

The smallest number of lesions, 6.77 to 9.15 lesions per 4 cm^2, was observed in the upper part of the plants (3rd leaf). In the three leaflets evaluated, the isolates ISO 4S and ISO 2A caused the greatest and smallest number of lesions in 4 cm^2, respectively.

Significant differences were observed in the number of lesions caused by *C. cassiicola* in the different soybean cultivars, considering the 1st ($P = 0.02$), 2nd ($P < 0.01$), and 3rd leaf ($P = 0.03$) (Figure 2).

For the 1st leaf, the smallest number of lesions (14.21 lesions in 4 cm^2) was observed in the cultivar BMX Potência RR, while the highest number of lesions (17.56 lesions in 4 cm^2) was observed in the cultivar BMX Elite IPRO. Intermediate numbers of lesions occurred in the other soybean cultivars.

The number of lesions on the 2nd leaf was lower for the cultivars SYN 1562 IPRO, NA 5909 RG, and BMX Potência RR, with 10.50, 10.80, and

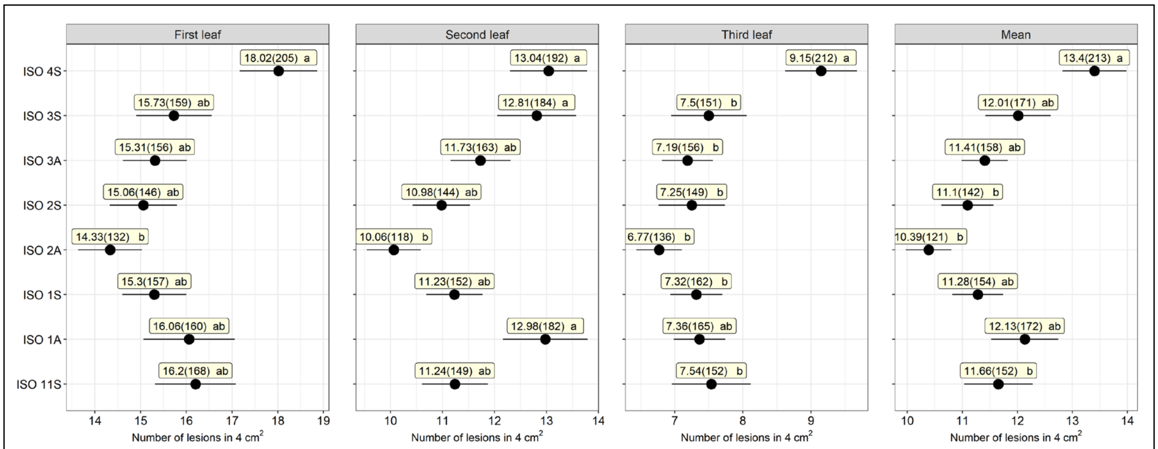

Figure 1 - Number of lesions in 4 cm^2 (mean (rank sum)) of the 1st, 2nd, and 3rd soybean leaves 12 days after inoculation with different isolates of *Corynespora cassiicola*. Different letters differ from each other by the Tukey test ($P \leq 0.05$).

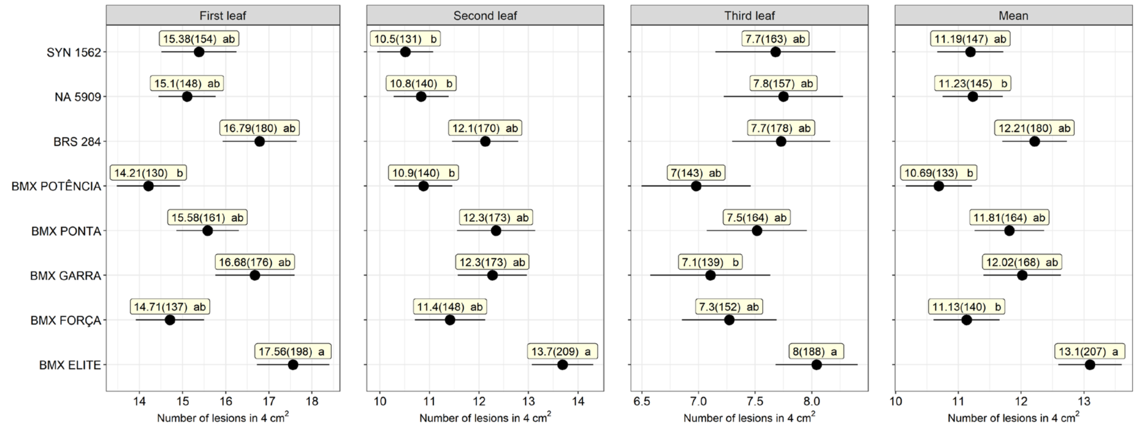

Figure 2 - Number of lesions in 4 cm² (mean (rank sum)) for the 1st, 2nd, and 3rd soybean leaves 12 days after inoculation with *Corynespora cassiicola* isolates in different soybean cultivars. Different letters differ from each other by the Tukey test ($P \leq 0.05$).

10.90 lesions per 4 cm², respectively, differing from the cultivar BMX Elite IPRO, in which the highest number of lesions (13.70 lesions per 4 cm²) was observed.

For the 3rd leaf, the lowest number of lesions (7.10 lesions per 4 cm²) occurred in the cultivar BMX Garra IPRO, differing from the cultivar with the highest number of lesions (8.00 lesions per 4 cm²), BMX Elite IPRO.

When considering the average number of lesions on the whole plant, it was possible to verify a lower occurrence of lesions, from 10.69 to 11.23 lesions in 4 cm², for BMX Potência RR, BMX Força RR, and NA 5909 RG, indicating less severity of the disease in these cultivars. Whereas, the highest number of lesions, 13.10 lesions per 4 cm², was observed in the cultivar BMX Elite IPRO, indicating lower lesion counts on these cultivars to target spot. Intermediate levels of severity occurred in the other cultivars (Figure 2).

Incubation period (IP)

No interaction occurred for incubation period (IP) between *C. cassiicola* isolate and soybean cultivar, for the 1st, 2nd, and 3rd leaflets ($P = 0.59$, 0.51, and 0.85, respectively). Significant differences in IP were verified for isolates of *C. cassiicola* inoculated on the 1st and 2nd leaf ($P < 0.01$), whereas no difference was verified for the 3rd leaf ($P = 0.12$). However, significant differences in the IP of *C. cassiicola* isolates among different soybean cultivars were found for the 1st leaf ($P = 0.03$) (Figure 3).

On the 1st leaf, the longest IP (5.05 days) was observed for the ISO 4S isolate, differing from ISO 1A, ISO 3A, and ISO 2A, which had the lowest IPs, ranging from 4.70 to 4.78 days. Similarly, for the 2nd leaf, the greatest IP (4.76 days) was observed for ISO 4S. However, the lowest IPs were observed for the ISO 11S and ISO 1S isolates, which presented 4.48 and 4.51 days, respectively. The IP of the different isolates of *C. cassiicola* varied from 4.20 to 4.45, 4.48 to 4.76, and 4.70 to 5.05 days, for the 3rd, 2nd, and 1st leaflets, respectively. Regarding cultivars, on the 1st leaf, the longest IP of *C. cassiicola* occurred for BMX Elite IPRO (5.05 days), while the lowest value was found in BRS 284, with an IP of 4.69 days. In the other cultivars, intermediate IPs were observed, ranging from 4.73 to 4.97 days. The IP of *C. cassiicola* in the different cultivars ranged from 4.23 to 4.46, 4.51 to 4.80, and 4.69 to 5.05 days, for the 3rd, 2nd, and 1st leaflets, respectively.

DISCUSSION

In this study, no interaction was observed between soybean cultivar and *C. cassiicola* isolate. Conversely TERAMOTO et al., (2013) reported significant interactions between Brazilian isolates of *C. cassiicola* and twelve soybean cultivars. However, different soybean cultivars and isolates were used in the previous research than used herein. Differences in the two studies, relative to cultivar versus isolate interactions, may be of interest to some, but seems irrelevant given how distinct the current study is from

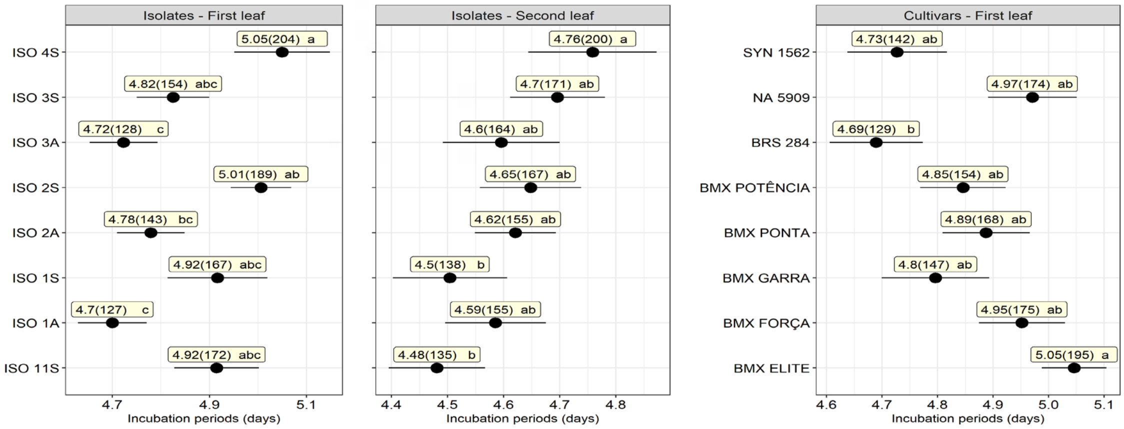

Figure 3 - Incubation periods on the 1st and 2nd soybean leaf inoculated with different isolates of *Corynespora cassiicola*, and incubation period 1st soybean leaves 12 days after inoculation with *C. cassiicola* isolates in different soybean cultivars in a climatized chamber (temperature of 26 ± 1 ºC, relative humidity of 80 ± 10 %, and photoperiod of 12 hours of light/dark). Different letters differ from each other by the Tukey test ($P \leq 0.05$).

the 2013 study. Lack of interaction may not have been reported because precision of measured variables and genetic variability in *C. cassiicola* isolates, which may be correlated with differences in pathogen effectors, pathogenicity, host specificity and host resistance for example.

Normally, for the germination of conidia of *C. cassiicola* isolates, temperatures of 10–35 °C (optimal 30 °C) induce greater fungus sporulation (FERNANDO et al. 2012). In soybean leaves, the conditions described that favor foliar infection by *C. cassiicola* are 80% free and/or relative humidity (RH), with a temperature between 20-30 °C (GODOY et al. 2015; MACKENZIE et al. 2018; ORTEGA-ACOSTA et al. 2020).

The non-similarity between this study and the one conducted by TERAMOTO et al. (2013) is probably due to the variability that the pathogen presents (DIXON et al. 2009). Isolates of *C. cassiicola* induced different lesion counts and incubation period (IP) depending on the soybean cultivar, corroborating results described by (DIXON et al. 2009), where isolates resulted in different levels of pathogenicity in soybean plants. In addition, Dixon's group reported genetic variability in the isolates. The continuous planting of susceptible cultivars allows great multiplication of *C. cassiicola*. Selection pressure carried out by different soybean production environments combined with the use of low-efficacy fungicides, has led to the selection of more aggressive isolates (XAVIER et al. 2013). This selection process contributes to the genetic variability that may result in differences in the pathogenicity observed in different soybean cultivars.

Quantifying the disease is considered one of the most important phases in a plant disease management program (BERGAMIN FILHO, 1976). Soybean cultivars more tolerant to target spot can be selected for future studies or even used in integrated disease management programs in several agricultural regions (SNOW et al. 1989; SINCLAIR et al. 1989; ALMEIDA et al. 2005). The smaller number of lesions may reflect greater resistance, as well as longer incubation periods. The cultivars that showed the least number of target spot lesions, NA 5909 RG, BMX Potência RR, and BMX Força RR, can be used by farmers in some regions, for example, South, Southeast and Midwest, along with the adoption of other management tactics to reduce the lesion counts. Evaluating the reaction of soybean cultivars to *C. cassicola* (MELO, 2009), only determined the number and diameter of lesions for the central leaf. In this study, the evaluations were carried out on the three leaflets, 1st, 2nd, and 3rd leaflets, in order to obtain more accurate data concerning the reaction of cultivars to target spot.

In addition to temperature, the position of leaflets can influence the incidence and expansion of target spot lesions in different pathosystems (ECKHARDT et al. 1984). In the current study, a higher incidence of lesions caused by the different *C. cassiicola* isolates occurred in the lower part of the plant, the 1st leaf, in all soybean cultivars tested. These results corroborate other studies that verified the occurrence of target spot in the lower stratum of plants (KUROZAWA et al. 2005). Also reported that (CONNER et al. 2013), under field conditions, the target spot appears initially in the lower stratum of plants, being the most affected part. The greater occurrence of the disease symptoms in the lower leaflets of soybean is due to the ideal microclimate formed in this stratum. Several authors reported that the fungus *C. cassiicola* can affect any stage of soybean crop. However, its incidence is more common after the canopy closes, because of a lower incidence of solar radiation and higher relative humidity, thus benefiting fungal development (LENTH et al. 2018; AMORIM et al. 2011). The incubation period is an important epidemiological variable, as it influences how fast the disease cycles occur (BERGAMIN FILHO, 2002; BERGAMIN FILHO, 1996). The IP of *C. cassiicola* isolates in different soybean cultivars varied from 4 to 5 days, under controlled conditions, with temperature of 26 ± 1ºC, relative humidity of 80 ± 10%, and photoperiod of 12 hours. For the same pathogen and plant species, (SEAMAN et al. 1965) reported that the symptoms of target spot appeared from 7 to 10 days in inoculated soybean plants with free moisture in the foliage, under greenhouse conditions. This difference in IP may be due to the different conditions the inoculated plants were subjected to, especially concerning temperature and photoperiod.

In this study, it was possible to verify that IP is lower in the upper leaf and higher in the lower leaf of soybean plants. The same pattern was found for *Pseudocercospora fuligena* in tomato plants in which 7-week leaflets had a higher pathogen IP compared to 1 to 5-week leaflets (MERSHA et al. 2014). Several plant species increase their resistance or tolerance to pathogens as they age. This phenomenon is known as age-related resistance (DEVELEY-RIVIÈRE et al. 2007). In agreement with other reported studies of similar pathosystems affecting soybean yield, demonstrate that younger common bean leaf tissues are more susceptible to the occurrence of *Uromyces appendiculatus* (FARBER et al. 2017). The lower IP or greater susceptibility may be related to the ability of the leaf tissue to synthesize defense compounds. As leaves age, phenols and proteins accumulate, constituting a chemical barrier that makes older tissues less prone to the development of pathogens (HUGOT et al. 2004). In the grapevine, younger leaflets were slightly more susceptible than older leaflets to powdery mildew infection; young susceptible leaflets were those that still acted as drain organs (STEIMETZ et al., 2012).

CONCLUSION

The study concludes that target spot severity and incubation period (IP) are influenced by the isolate of *C. cassiicola*, soybean cultivar, and leaf position in the plant. Isolates of *C. cassiicola* can cause higher (ISO 4S) or lower (ISO 2A, ISO 2S, and ISO 11S) severity of target spot. There are no soybean cultivars resistant to target spot; however, less lesion counts occurs in the cultivars BMX Potência RR, BMX Força RR, and NA 5909 RG, while greater susceptibility is verified for the cultivar BMX Elite IPRO. In addition, the longest incubation period (IP) for the evaluated *C. cassiicola* isolates occurs in the cultivar BMX Elite IPRO and the shortest in BRS 284. Regardless of the cultivar evaluated, the IP tends to be longer for the ISO 4S isolate. The different isolates of *C. cassiicola* cause more lesions in the lower leaf and fewer lesions in the upper leaf of the plants. A similar pattern occurs for IP, with a longer period of incubation in the lower leaf and a shorter period in the upper leaf of soybean plants.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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