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Characterization and Incidence of Target Spot Lesions in Unifoliate Leaves, Petioles, and Stems of Soybean Cultivars

Jacqueline Dalbelo Puia[1], Ana Maria da Silva Moreira[3], Adriano Thibes Hoshino[1], Humberto Godoy Androcioli[2], Estela Mariani Klein[1], Marcus Eugênio Oliveira Briozo[3], Sandra Cristina Vigo[3] & Marcelo Giovanetti Canteri[1]

[1] Agronomy Department, Universidade Estadual de Londrina, Londrina, PR, Brazil

[2] Department of Entomology, Instituto de Desenvolvimento Rural do Paraná, Londrina, PR, Brazil

[3] Department of Phytopathology, Instituto de Desenvolvimento Rural do Paraná, Londrina, PR, Brazil

Correspondence: Jacqueline Dalbelo Puia, Agronomy Department, Universidade Estadual de Londrina, Londrina, PR, Brazil. Tel: 55-43-3376-2114. E-mail: puia.agro@gmail.com

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Abstract

This study aimed to classify the different types of leaf lesions caused by target spot and quantify their incidence in plant tissues of soybean cultivars infected by *Corynespora cassiicola* isolates. The experiment was conducted in a randomized block design in an 8 × 8 factorial arrangement, consisting of eight *C. cassiicola* isolates and eight soybean cultivars. Soybean plants were inoculated by spraying fungal suspension on the leaflets at a concentration of 2×10^4 conidia mL^{-1}. Target spot lesions were classified, assigning scores for each type of symptom observed. The incidence of lesions in plant tissues was evaluated 10 days after inoculation, recording the presence of lesions. Five patterns of lesions were observed, ranging from small (0.74 mm) to large (9.30 mm) necrotic spots. Symptoms capable of causing defoliation occurred in BMX Elite IPRO, BRS 284, BMX Garra IPRO, and Nidera 5909 RG when inoculated with ISO 1S, ISO 4S, and ISO 11S. The highest frequency of lesioned trefoils was verified in the upper and lower (3rd and 1st trefoil) strata of soybean plants. Lesions were detected in cotyledons, unifoliate leaves, petioles, and stems of plants from all cultivars evaluated in this study. The isolate ISO 4S caused higher incidence of lesions in petioles compared with ISO 2A and ISO 2S. Moreover, ISO 4S produced more lesions in the stems of BMX Potência RR and BMX Force RR than BMX Elite IPRO. The incidence of petiole lesions caused by *C. cassiicola* increased as the petiole insertion height into the main stem decreased.

Keywords: *Corynespora cassiicola*, *Glycine max*, plant tissues, incidence of lesions

1. Introduction

The target spot, caused by the fungus *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei 1950, has become an important foliar disease that affects soybean production in Brazil and worldwide (Farr & Rossman, 2019). The fungus survives in infected seeds and crop debris, mainly in fallow for at least two years (Almeida et al., 2001), acting as a primary source of inoculum for new epidemics. The pathogen can also survive in stems and roots in the form of chlamydospores (Oliveira et al., 2012).

Seed treatment, foliar spray with fungicides, and crop rotation are the most applied control strategies for disease management (Almeida et al., 2005). However, target spot epidemics have been frequent in many soybean-growing regions in Brazil due to the absence of resistant cultivars and fungicide-resistant isolates (Godoy et al., 2012; Teramoto et al., 2013).

Furthermore, leaf infections are favored by the presence of free water in the leaves and relative humidity above 80% (Snow & Berggren, 1989). Under this favorable environment, a germ tube is formed, with subsequent penetration into the host tissue (Amorim & Pascholati, 2011). The pathogen releases a toxin called cassiicolin into the host plant, which kills adjacent tissues at the site of infection. This toxin is essential for the initial establishment and pathogenicity of the fungus (Breton et al., 2000). In environments with high humidity and temperatures of 22 to 30 °C, symptoms appear 5-7 days after infection (Amorim & Pascholati, 2011). Typical leaf symptoms of target spot are small circular brown lesions with a well-developed yellow halo. Coalescence of lesions occurs as spots expand (Almeida et al., 2005).

In addition to symptoms in leaf tissue, the fungus can cause root and pod rot, stem lesions, and seed infection (Raffel et al., 1999). However, the pathogen is a typical representative of the group that reduces leaf photosynthetic area due to the induced symptoms, accelerating the natural process of leaf senescence and negatively affecting productivity (Boote et al., 1983; Bedendo, 1995). For instance, Godoy et al. (2016) reported a reduction in soybean yield by up to 35% in cultivars susceptible to target spot, while Molina et al. (2019) mentioned losses of up to 40% in yield.

According to Mesquini (2012), target spot severity can affect up to 37% of the lower stratum of plants without decreasing production in a susceptible cultivar owing to the low contribution of this stratum in seed formation and filling. However, the disease reduces yield when it occurs in the plant middle stratum, as this area presents high light interception and a significant contribution to seed formation and filling (Sakamoto & Shaw, 1967).

It is believed that target spot symptoms may vary depending on the *C. cassiicola* isolate and the host plant. This variation can result in greater or lesser amount of affected leaf area, influencing the speed of leaf senescence and consequently damaging production. Furthermore, lesions present in petioles can also result in anticipation of leaf senescence, with consequent defoliation. Despite the importance of this information, studies that classify and describe how symptoms progress in soybean plants are scarce. Therefore, the present study aimed to classify the different types of leaf lesions of soybean target spot and quantify their incidence in commercial soybean cultivars infected by different *C. cassiicola* isolates.

2. Method

2.1 Soybean Cultivars

The commercial soybean cultivars Brasmax Elite IPRO, Brasmax Força RR, Brasmax Garra IPRO, Brasmax Ponta IPRO, Brasmax Potência RR, BRS 284, Nidera 5909 RG, and SYN 1562 IPRO were selected for presenting some level of susceptibility to target spot.

Seeds of the chosen cultivars were germinated in filter (germitest®) paper, and after five days, seedlings were distributed in 1-L pots containing sieved soil (Oxisol Red dystroferric). Five (5 g) of the granulated chemical fertilizer 4-30-10 (N-P_2O_5-K_2O) were added to each pot. After planting, the pots were kept in a greenhouse until the time of inoculation with *C. cassiicola* isolates.

2.2 C. cassiicola Isolates

Eight isolates of *C. cassiicola* from monosporic culture, deposited in the collection of the Seed Pathology laboratory of the Rural Development Institute of Paraná (IDR-Paraná) were used. The isolates were obtained from plant lesions of cotton (ISO 1 A, ISO 2 A, and ISO 3 A) and soybean (ISO 1 S, ISO 2 S, ISO 3 S, ISO 4 S, ISO 11 S) collected in the field in the 2018/19 harvest.

C. cassiicola was multiplied in 50 Petri dishes (Ø = 9 cm) containing V8 Juice culture medium (5 g L^{-1} calcium carbonate, 340 mL V8 juice, 34 g L^{-1} of agar) with streptomycin (50 mg L^{-1}). The plates were kept at 25±2 °C and 12/12 hours photoperiod for 10 days. After this period, *C. cassiicola* colonies showed abundant sporulation and were used as inoculum.

2.3 Experimental Design

The experiment was performed using the randomized block design in an 8 × 8 factorial scheme (8 *C. cassiicola* isolates × 8 soybean cultivars). The blocks were formed over time. The experimental unit consisted of two pots containing one plant each.

2.4 Inoculation Conditions

C. cassiicola isolates were inoculated when soybean cultivars were at the phenological stage V4, characterized by the third fully developed trifoliate leaf (Neumaier et al., 2000). To stimulate the opening of the plant stomata, each pot received 1 g of urea (CH_4N_2O) 24 hours before inoculation.

For each *C. cassiicola* isolate, a suspension of conidia was prepared at a concentration of 2×10^4 conidia mL^{-1}, in which one drop per liter of the adhesive spreader Tween 20 and 0.4 g of unflavored gelatin were added. These adjuvants were added to improve the distribution of the inoculum suspension and adhesion on soybean leaflets during inoculation. The conidia suspensions were inoculated in the coolest period of the day on soybean leaves until the point of drainage, using a manual sprinkler.

In order to favor pathogen infection, after inoculation, pots were transferred to a climatized chamber with 26±1 °C, relative humidity of 85 to 90%, and a 12/12 hours light/dark photoperiod, remaining in these conditions for 48 hours. After this period, the plants were randomly distributed inside the climatized chamber and kept under the same temperature and photoperiod conditions and relative humidity of 80±10% until the end of the evaluations at 20 days after inoculation (DAI).

2.5 Evaluations

The evaluations were carried out in the three leaflets of the 1st, 2nd, and 3rd trefoils, totaling nine leaflets per plant. All leaflets evaluated were fully expanded at the time of inoculation. The 1st, 2nd, and 3rd trefoils are located in the lower, middle, and upper strata of soybean plants in stage V4, respectively.

For each isolate × cultivar combination, class and influence of trefoil position on target spot lesions in soybean leaflets, disease incidence, and frequency in different plant tissues were evaluated.

The classification of target spot lesions in leaflets was performed 12 days after inoculation by a score scale elaborated at the time of evaluation with the types of lesions observed in soybean cultivars. From this information, the frequency of lesion class according to the trefoil position in plants was quantified.

To quantify the incidence of target spot lesions in different plant tissues, the presence and/or absence of lesions in unifoliate leaves, petioles, stems, and cotyledons in soybean cultivars was recorded. From this information, the frequency of plant tissues with lesions was estimated. The incidence of lesions was quantified 10 days after inoculation.

2.6 Statistical Analysis

To classify the lesions, a descriptive analysis of the disease was performed. Incidence data were subjected to analysis of variance (ANOVA); when significant differences were found, means were compared using the Tukey test ($p < 0.05$). The data were subjected to the assumptions of normality of errors and homogeneity of variances by the Shapiro-Wilk and Bartlett tests ($p > 0.05$), respectively. Statistical tests were performed using the SISVAR software.

3. Results

3.1 Lesion Classification

Five classes of leaf symptoms caused by different *C. cassiicola* isolates in soybean cultivars were observed. The classes of symptoms identified ranged from score 1, presenting small necrotic spots (0.74 mm) with little evident chlorotic halos, to 5, showing large necrotic spots (9.30 mm) with large chlorotic halos (Figure 1).

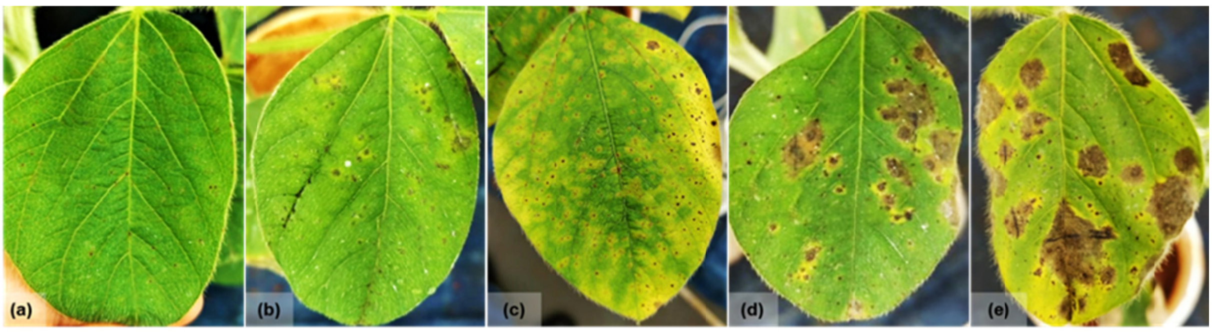

Figure 1. Classifications of symptoms present in leaflets of soybean cultivars inoculated with *C. cassiicola*: (a) 1 = small necrotic spots (0.74 mm) with little evident chlorotic halos; (b) 2 = small necrotic spots (1.80 mm) with evident chlorotic halos; (c) 3 = small necrotic spots (2.35 mm) with large chlorotic halos; (d) 4 = large necrotic spots (6.30 mm) with evident chlorotic halos; (e) 5 = large necrotic spots (9.30 mm) with large chlorotic halos. Experiment carried out in a climate-controlled chamber (T = 25±2 ºC; RH% = 85 to 90; photoperiod 12/12)

The highest percentages of trefoils with lesions capable of leading to defoliation were provided by the isolates ISO 4S, ISO 1S, and ISO 11S, with values (sum of scores equal to or above 3) of 71, 60, and 42%, respectively (Figure 2). The greatest percentages of trefoils with scores 1 and 2 were observed for ISO 3S (69%), ISO 2S (77%), ISO 3A and ISO 1A (both with 81%), and ISO 2A (94%) (Figure 2).

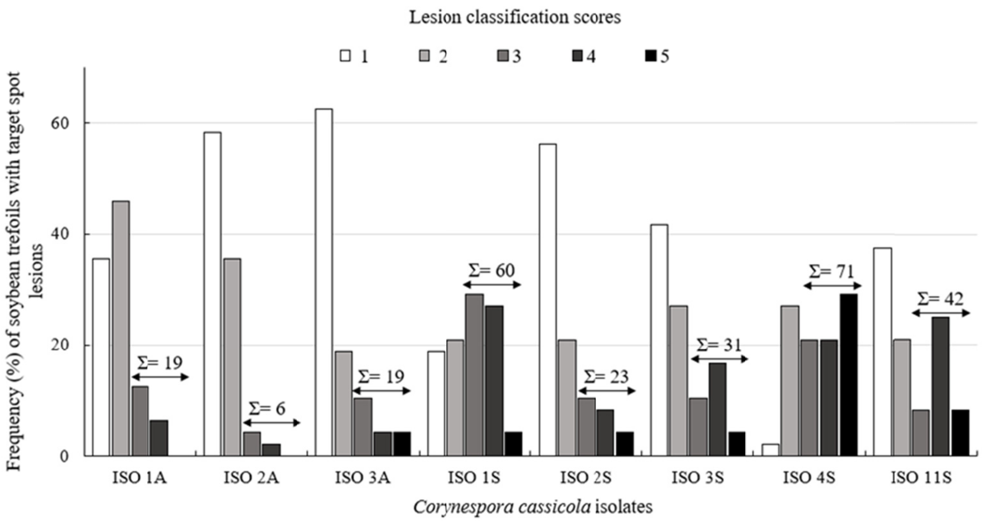

Figure 2. Relative frequency of scores 1 to 5 corresponding to target spot symptoms in soybean trefoils after 12 days of inoculation with different *C. cassiicola* isolates. Σ: sum of trefoils with scores equal to or above 3

Differences in classes of target spot lesions were observed among soybean cultivars. The highest frequencies of trefoils with lesions capable of causing defoliation, scores equal to or above 3, were observed in the cultivars BMX Elite IPRO, BRS 284, BMX Garra IPRO, and Nidera 5909 RG (44, 42, 42, and 38%, respectively) (Figure 3). For BMX Força RR, BMX Potência RR, BMX Ponta IPRO, and SYN 1562 IPRO, the scores 1 and 2 predominated, representing 69 to 85%.

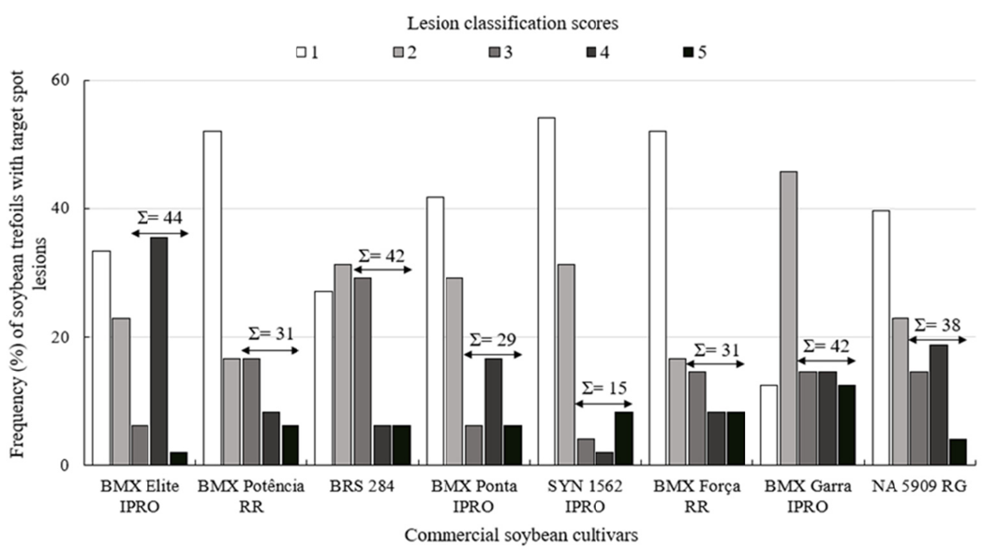

Figure 3. Relative frequency of scores (1 to 5) corresponding to target spot symptoms in soybean trefoils after 12 days of inoculation with different soybean cultivars. Σ: sum of trefoils with scores equal to or above 3

3.2 Influence of Trefoil Position on Lesion Classification

Differences in the frequency of lesioned trefoils caused by *C. cassiicola* were observed among the lower, middle, and upper strata of soybean plants (Figure 4). The highest frequency of lesioned trefoils, with defoliation potential (scores equal to or above 3), was verified for the upper (3rd trefoil) and lower (1st trefoil) strata of the plants, with 43 and 40%, respectively. On the other hand, the middle stratum of the plants (2nd trefoil) had a predominance of scores 1 and 2, with an accumulated frequency of 81%.

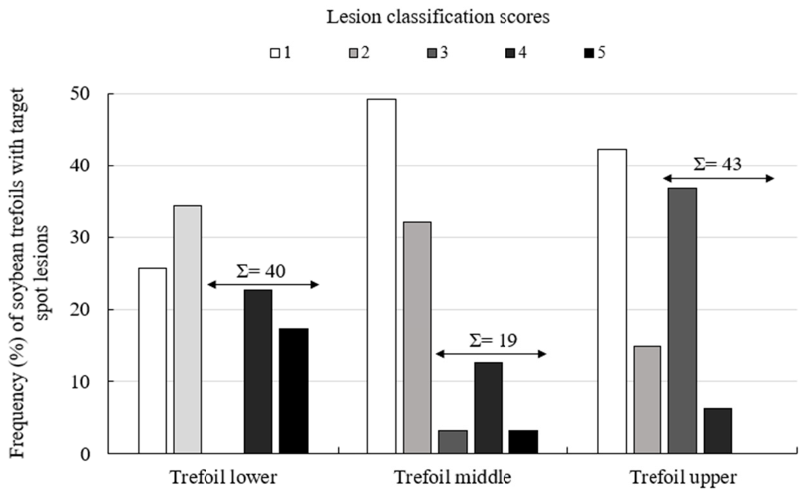

Figure 4. Relative frequency of scores (1 to 5) corresponding to target spot symptoms in soybean trefoils, 12 days after inoculation with different isolates of *C. cassiicola*. Σ: sum of trefoils with scores equal to or above 3

3.3 C. cassiicola Infection in Different Plant Tissues

In addition to occurring in leaves, target spot lesions were verified in the unifoliate leaves, petioles, stem, and cotyledons of soybean. In unifoliolate leaves, symptoms started with small brown spots with a yellowish halo. After four days, they evolved to large light brown circular spots (Figure 5a). Finally, a very intense yellow halo circled the necrotic area, with subsequent senescence and leaf drop.

In the petioles and stems of soybean plants, the lesions had a depressed and elongated appearance with a reddish-brown color. These lesions progressed along the plant in a longitudinal direction, where coalescence might have occurred (Figures 5b and 5c).

Five days after inoculation, the presence of *C. cassiicola* mycelium and abundant sporulation were verified in soybean cotyledons, regardless of the cultivar. The cotyledons adhered to the plant or broke off and fell to the ground. Cotyledons were collected (Figures 5d and 5e).

In general, soybean plants inoculated with *C. cassiicola* isolates showed lesions that ranged from small necrotic spots without a yellowish halo to large necrotic spots with evident chlorotic halos (Figure 5f). In addition, the presence of lesions was verified in the adaxial and abaxial parts of the leaves.

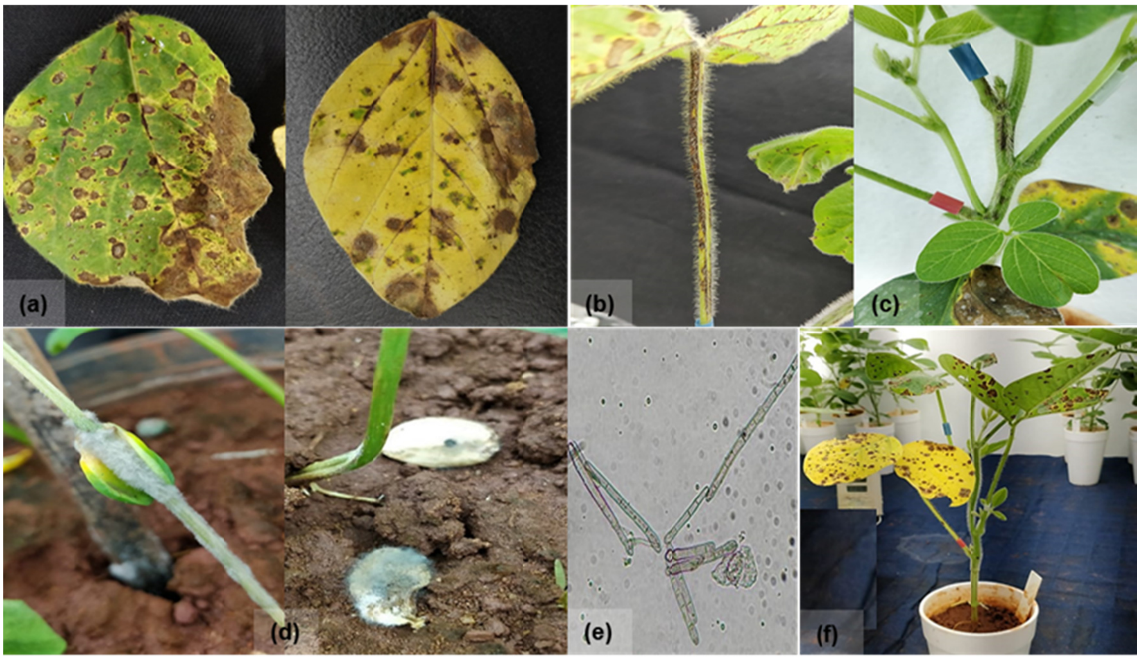

Figure 5. Target spot symptoms caused by the fungus *C. cassiicola* on soybean plants. (a) Unifoliate leaves with target spot lesions. (b) Dark and depressed lesions on the petiole and (c) stems. (d) Cotyledons with the presence of fungal mycelium. (e) Conidia obtained from cotyledons with sporulation observed on slides under an optical microscope. (f) Plant with symptoms of the disease

3.4 Frequency of C. cassiicola Infection in Different Plant Tissues Organs

No interaction between *C. cassiicola* isolate and soybean cultivar was detected in relation to the presence of lesions on unifoliate leaves, petioles, and stems. Likewise, no significant difference was found for frequency of lesions on unifoliate leaves within *C. cassiicola* isolates ($p = 0.06$) and within soybean cultivars ($p = 0.54$). A frequency of lesions in unifoliate leaves above 96% was verified (Figure 6).

Significant differences were also found among *C. cassiicola* isolates regarding the frequency of lesions on petioles ($p = 0.02$) and for soybean cultivars in relation to the frequency of lesions on stems ($p = 0.02$). The frequency of petioles with lesions was high, ranging from 80 to 96%. However, the ISO 4S isolate, which caused 96% of petioles with lesions, differed from ISO 2A and ISO 2S, which resulted in the lowest frequencies of petioles with lesions, 81 and 80%, respectively.

The other isolates provided intermediate frequencies, presenting values from 93 to 84% of petioles with lesions. It was not possible to verify differences in the frequency of petioles with lesions among soybean cultivars (Figure 6).

Regarding the frequency of lesions on the stem, differences were found among soybean cultivars. Higher frequencies of stems with lesions (97%) were observed in the cultivars BMX Potência RR and BMX Força RR, differing from BMX Elite IPRO, which had the lowest frequency of stems with lesions (78%). No differences between *C. cassiicola* isolates were detected regarding their ability to cause lesions in soybean stems.

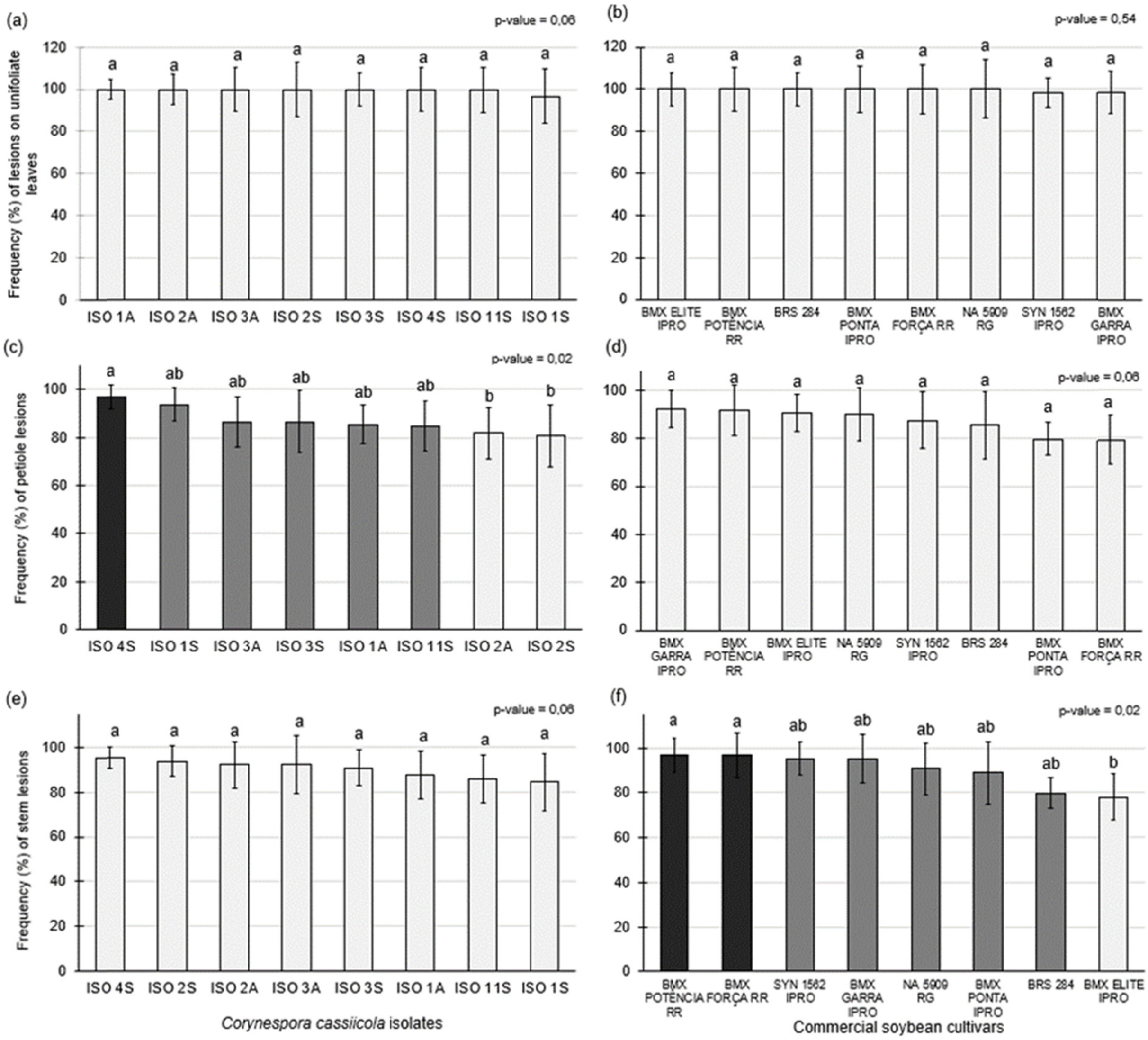

Figure 6. Frequency of plant tissues of soybean cultivars with lesions caused by different *C. cassiicola* isolates after 12 days of inoculation

As for the presence of target spot lesions on petioles, 96% of petioles on the 1st trefoil contained lesions, differing from the 3rd trefoil, with 77% of lesioned petioles. For petioles of the 2nd trefoil, lesions were found in a frequency of 90% (Figure 7).

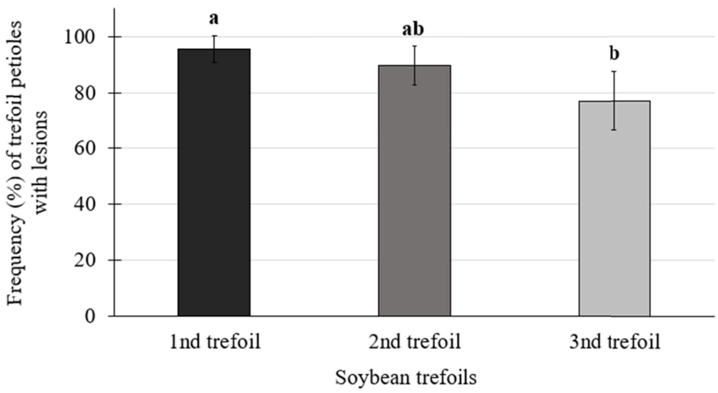

Figure 7. Frequency of petioles with lesions caused by *C. cassiicola* in relation to trefoil position in soybean, after 12 days of inoculation

4. Discussion

In this study, no interaction was observed between soybean cultivar and *C. cassiicola* isolate. Differences were observed in the classification of lesions produced by the isolates in different soybean cultivars. The lesions were distributed in five classes, from small necrotic spots with little evident chlorotic halos to large necrotic spots with large chlorotic halos. The patterns of symptoms caused by *C. cassiicola* corroborate the results of Henning et al. (2014), who described that symptoms on soybean leaves range from necrotic lesions, brown spots to large circular dark brown spots, up to 2 cm in diameter, with a yellow margin and a dark spot in the center surrounded by darker concentric rings.

C. cassiicola isolates show varying patterns of lesions. Almeida et al. (2005) also characterized target spot symptoms as small reddish-brown to brown spots, evolving into rounded or irregular-shaped spots of the same hue. The symptoms described by the authors coincide with the characteristics found in our study. However, we identified greater variation of symptoms, probably due to the use of different soybean isolates and cultivars.

The cultivars that presented lower target spot lesion severities, BMX Força RR, BMX Potência RR, BMX Ponta IPRO, and SYN 1562 IPRO, expressed some level of resistance to the disease, showing patterns of small lesions. According to Lamari and Bernier (1989), the type of lesion expressed in the plant may be related to resistance. For example, small lesions that do not increase in size occur in more resistant plants, whereas dark spots surrounded by extensive necrosis and chlorosis that may involve the entire leaf indicate the susceptibility of the host.

Therefore, large lesions with extensive chlorosis may indicate that the plant is somewhat susceptible to the disease. This pattern occurred for BMX Elite IPRO, BRS 284, BMX Garra IPRO, and Nidera 5909 RG cultivars. Sache et al. (1993) described that small lesions are an indication that the inoculum or disease efficiency was high, while some large lesions result from low inoculum and high susceptibility of the plant or cultivar.

Another critical factor in the pathogen-host interaction and classes of symptoms observed is the production of enzymes and toxins. Many phytopathogenic fungi secrete enzymes during the growth and pathogenesis process (Thormann et al., 2000). This fact indicates a strong association regarding the pattern of symptoms and the toxin of *C. cassiicola* isolates. Annakutty (1998) demonstrated that *C. cassiicola* produces a toxin called cassiicolin, responsible for the infection process and the appearance of symptoms.

Once inside the host plant, the pathogen *C. cassiicola* releases cassiicolin, killing adjacent tissues at the infection site, colonizing necrotic tissues and reproducing. Barthe et al. (2007) demonstrated that cassiicolin behaves as a host-specific toxin, sharing the same host range as the pathogen from which it originated.

Higher frequencies of target spot lesions occurred in the upper and lower trefoils, 3rd and 1st trefoils, with lesions capable of causing defoliation. According to Upchurch and Ramirez (2010), plants respond to infections by pathogens through defense mechanisms, which include the synthesis of antimicrobial compounds, such as phenols and phytoalexins, production of reactive oxygen species, and tissue lignification. Therefore, perhaps this factor is related to the leaf capacity to express greater or lesser defense reaction levels. Moreover, phytoalexins may be involved in the development of spots on soybean leaflets. Braga (2008) reported that phytoalexins are antimicrobial defense compounds synthesized and accumulated in plants after contact with microorganisms.

The ability of soybean plants to reduce fungus colonization depends on a combination of different defense mechanisms. These defense mechanisms vary according to the host-pathogen interaction (Hammond-Kosack & Parker, 2003). Fortunato et al. (2015) described, in the early stages of *C. cassiicola* infection, an increase in polyphenol oxidase activity, concentrations of total soluble phenolics and lignin, and lower lipoxygenase activity associated with soybean resistance to target spot.

Several factors can influence plant resistance to infection by pathogens. Plant developmental stage, known as age-related resistance or ontogenic resistance, is among them. It is related to the ability of whole plants or plant parts to resist or tolerate disease as they age and mature. Age-related resistance occurs in many plant species and is generally broad-spectrum (Devley-Rivière et al., 2007). For example, in grapevines, the older leaves are more resistant to powdery mildew than the younger ones (Steimetz et al., 2012).

Owen et al. (2013) described that the most significant soybean yield loss results from the defoliation of the upper stratum of plants compared to stages R3 and R5 of the lower stratum of plants. Considering the predominance of lesions in the upper trefoil leaflets, representing younger and more tender leaf tissues, management measures should be taken so that these younger leaves present greater resistance to the pathogen.

As for *C. cassiicola* incidence in soybean tissues, fungus-derived lesions can be observed on petioles, stems, unifoliate leaves, and cotyledons, in addition to leaves. The symptoms coincide with those Kurt (2004) reported in tomato plants infected with *C. cassiicola*, presenting brownish and elongated spots. The spots visualized on the petioles and stems of plants were mainly dark brown lesions and elongated spots slightly depressed.

In addition to the symptoms, fungus reproductive structures were observed on both sides of unifoliate leaves, coinciding with the results found by Mello (2009), where fungus spores were produced on both sides of leaves but less abundantly on the abaxial side. Another vegetative organ that showed symptoms and signs of reproductive structures of the fungus was the cotyledon. Almeida et al., (2005) and Campos et al., (2008) described that *C. cassiicola* is disseminated through the production of conidia from primary lesions in cotyledons and hypocotyl which can contribute to the increase of inoculum in the area.

The incidence of lesions in the petiole increased as the height of petiole insertion on the main stem decreased, that is**,** the first trefoil was more attacked by the disease. Develey-Rivière et al. (2007) reported that infection efficiency is related to the development stage of the host tissue in many plant-pathogen interactions. Thus, depending on the stage, pathogen development and plant tissue susceptibility may increase.

The resistance mechanisms involved in canopy position-related resistance, still a matter of speculation, differ from those involved in the response to infection in classical defense systems (Gee et al., 2008). Our study demonstrated greater sensitivity to infections by *C. cassiicola* in the upper and lower strata of soybean plants, which could significantly affect yield.

5. Conclusions

Symptoms caused by *C. cassiicola* vary according to isolate and soybean cultivar. In this study, five patterns of target spot lesions were identified, ranging from small necrotic spots (0.74 mm) with little evident chlorotic halos to large necrotic spots (9.30 mm) with large chlorotic halos. Symptoms capable of causing defoliation were frequent for the soybean isolates evaluated in the cultivars BMX Elite IPRO, BRS 284, BMX Garra IPRO, and Nidera 5909 RG. Soybean middle-strata trefoils (2nd trefoil) presented lower target spot severity. Moreover, we identified target spot lesions in cotyledons, unifoliate leaves, petioles, and stems of plants from the evaluated soybean cultivars. Finally, lesion incidence in petioles increases as the height of petiole insertion in the main stem decreases.

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