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Impact of Ultraviolet-C Exposure and Mechanical Stress on Conidium Production in *Corynespora cassiicola* Isolates From Cotton and Soybean

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Abstract

The procurement of inoculum for bioassays with target spot, a significant disease in cotton and soybeans, caused by *Corynespora cassiicola* can be hindered by the low production of conidia on artificial culture media. The study aimed to determine whether mechanical stress on the mycelium and exposure to ultraviolet-C (UV-C) radiation for varying durations could enhance conidial production in *C. cassiicola* isolates. Eight isolates from cotton and soy were used, grown in V8 juice medium. After five days of incubation in a climate-controlled chamber, each isolate either underwent mycelium scraping or remained unscraped and was subsequently exposed to UV-C radiation for either 1.0, 1.5 or 2.0 min, in comparison to a control group with zero UV exposure time. Following these procedures, conidial suspensions from each isolate were obtained and quantified (conidia per mL) using a Neubauer chamber The ISO 3S and ISO 4S isolates were found to produce more conidia than the other isolates, regardless of whether they were subjected to mycelium scraping or exposure to UV-C radiation. For most isolates, exposure to UV-C radiation for 1.0-1.5 min led to increased conidium production. Generally, it was not feasible to discern differences in conidial production with respect to the mycelium scraping process. Nevertheless, exposure to UV-C radiation for 1.0 min can be used to induce conidium production in *C. cassiicola* isolates.

Keywords: mycelium scraping, number of spores, radiation time, target-spot

1. Introduction

The target spot, caused by the fungus *Corynespora cassiicola* (Berk & MA Curtis) infects leaf tissues, petioles, pods, seeds, stems, hypocotyls, and roots of plants (Hartman et al., 1999; Puia et al., 2022). The disease is reported in countries with tropical and subtropical climates, affecting over 530 species across 400 genera of plants, including monocots and dicots of agricultural significance (Oliveira et al., 2012; Sumabat et al., 2018a; Farr & Rossman, 2020; Godoy et al., 2020).

The following plants have been reported to be affected by the disease: acerola (Silva et al., 1997), cotton (Sumabat et al., 2018b), lettuce (Santos et al., 2007), beans (Mendes et al., 1998), papaya (Yan-Xiang et al., 2011), cucumber (Cutrim & Silva, 2003), bell pepper (Shimomoto et al., 2008), soy (Almeida et al., 2005), rubber tree (Yan-Xiang et al., 2011), and tomato (Lopes & Ávila, 2005). Weed species such as Brazilian ironweed (*Vernonia polyanthes*), lantana (Barreto et al., 1995), and dayflower (*Commelina bengalensis* L.) (Cutrim & Silva, 2003; Oliveira et al., 2007) can also be infected.

Previously regarded as a minor disease in cotton and soybean cultivation, target spot has reemerged as an endemic disease in recent decades due to the rise of monoculture, the use of susceptible cultivars, and reduced sensitivity of the pathogen to fungicides (Molina et al., 2022; Xavier et al., 2021; Mello et al., 2022). In cotton and soybean crops, yield reductions of up to 42% have been reported in susceptible cultivars (Molina et al., 2019).

The primary strategies for managing *C. cassiicola* include the use of fungicides and the cultivation of disease-tolerant or resistant cultivars (Godoy et al., 2020). Thus, acquiring conidia from the fungus *C. cassiicola* is

an essential factor in studies aimed at selecting cultivars that are less susceptible to the disease, as well as in fungicide efficacy tests that control the pathogen (Almeida et al., 2005; Mello et al., 2018). Therefore, it is important to encourage the production of conidia in *C. cassiicola* isolates.

Many *C. cassiicola* isolates exhibit low conidial production in artificial culture media, which complicates inoculation across laboratory, greenhouse, and field conditions (Beckman et al., 1983; Mello et al., 2018). The composition of the culture medium determines the quality and quantity of mycelial growth and subsequent sporulation of *C. cassiicola* isolates, which can be cultivated on media such as potato dextrose agar, malt agar, and Czapek agar. However, enhanced growth and sporulation are observed in V8 juice agar medium (Gava, 2002; Melo & Reis, 2010; Puia et al., 2021).

Even when adjusting the culture medium to enhance conidial production, obtaining conidial suspensions is not straightforward. In most studies, the inoculum of *C. cassiicola* is prepared by macerating mycelia with the culture medium in distilled water, followed by straining through gauze to obtain the suspension that is used to inoculate the plants (Sobers, 1966; Spencer & Walter, 1969; Dixon et al., 2009). Throughout this process, a large portion of the conidia are retained along with the mycelium in the gauze. Additionally, a significant amount of nutrients from the culture medium is incorporated into the inoculum, which diminishes the virulence of the fungi and, consequently, the establishment of the disease in the plants under study (Banttari & Wilcoxson, 1964).

The highest production of *C. cassiicola* conidia could be achieved by increasing the amount of fungal culture in the growth medium; however, the components of the culture medium are costly and requires a greater number of Petri dishes in the production process. In this way, it is desirable to enhance the efficiency of conidia production using fewer Petri dishes and less culture medium.

Factors such as light quality and intensity can influence growth, pigmentation, conidia formation induction, and germination (Minussi et al., 1997), as well as the shape and size of the conidia in many fungal species (Mathur & Neergaard, 1973). Similar to plants, fungi exhibit a circadian rhythm, with the ability to discern day from night, due to a wide array of photoreceptors within their cells that are sensitive to a broad spectrum of electromagnetic waves, ranging from ultraviolet to infrared (Purschwitz et al., 2006; Dong et al., 2013; Ohm et al., 2013).

Specifically, ultraviolet light can affect the metabolism, growth, and reproduction of most fungi (Cochrane, 1958; Idnurm & Heitman, 2005). However, the mechanisms involved in fungi's perception of and response to light are not yet fully understood (Idnurm & Heitman, 2005).

As of yet, there is a lack of studies indicating the influence of light on the production of conidia in *C. cassiicola*. Understanding the proper conditions for cultivating and producing conidia of a microorganism *in vitro* is crucial, providing a solid foundation for various studies, including strategies for target spot control, and rendering a better understanding of environmental factors in epidemic development (Kranz & Hau, 1980; Leite & Amorim, 2002; Teramoto et al., 2013).

Therefore, the aim of the study was to develop a method to induce conidium production in *C. cassiicola* from various cotton and soybean isolates through mechanical stress of the fungal colonies and exposure time to ultraviolet-C (UV-C) radiation.

2. Material and methods

2.1 Experimental Setup

The experiment was conducted at the Seed Pathology Laboratory of the Rural Development Institute of Paraná, IAPAR-EMATER (IDR-Paraná), in Londrina-PR, using *C. cassiicola* (*Cc*) isolates from various regions of Brazil.

The isolates ISO 1C (Sertanópolis-PR), ISO 2C (Jataizinho-PR), and ISO 3C (Porecatu-PR) were obtained from cotton lesions, while ISO 1S (Goiânia-GO), ISO 2S (Arapongas-PR), ISO 3S (Sorriso-MT), ISO 4S (Londrina-PR), and ISO 11S (Diamantino-MT) were obtained from soybean plants collected in commercial crops during the 2018/19 crop season.

The isolates were grown on Petri dishes (Ø = 9 cm) filled with V8 juice medium and incubated in a BOD-type germination chamber, at a temperature of 25±1 °C with a 12/12 h light/dark photoperiod.

2.2 UV-C Radiation Exposure

After a five-day incubation period, conidium production for each of the eight isolates was triggered with or without mycelium scraping. The isolates were then subjected to four different UV-C radiation exposure durations (0, 1, 1.5, and 2 min), in a completely randomized design with an 8 × 2 × 4 factorial arrangement, and five replicates for each factor combination. Each repetition consisted of a Petri dish containing the respective fungal isolates.

The stress on the mycelia of each isolate was induced by scraping the colonies using a microscopy blade. After this process, the plates were exposed with covers, arranged in a single layer (not stacked) at a distance of 60 cm from the UV-C radiation-emitting lamps (254-280 nm), with a power of 30 W, inside a Buzatto's® laminar flow cabinet. The laminar flow hood lights were turned on 30 min prior to the start of the experiment to establish a stable level of irradiation over the samples. Subsequently, the plates were once again placed in a BOD-type germination chamber for 24 h, under the same temperature and photoperiod conditions previously mentioned.

2.3 Conidial Suspension and Analysis

After the 24 h incubation period, conidial suspensions from the isolates were obtained by washing the Petri dishes with 10 mL of autoclaved distilled water. The mycelium was separation through filtration using gauze, the conidia from the suspensions were quantified using a Neubauer chamber under an optical microscope (400x), and the concentration was expressed in conidia per mL.

The assumptions of error normality and variance homogeneity were tested using the Shapiro–Wilk and Levene tests, respectively. Once these assumptions were met, the concentration of conidia among the isolates and induction methods were compared using the analysis of variance followed by the Tukey's test at a 5% significance level. The analysis also included linear and non-linear regression (quadratic or the Bragg model). All analyses were conducted using the R software (Team, 2021).

3. Results

3.1 Effect of UV-C Radiation on Conidial Production in Cc Isolates

The exposure time to UV-C radiation was found to affect the number of conidia produced by the different isolates of *C. cassiicola*, except for isolates ISO 2C and ISO 3C that were subjected to the mycelium scraping procedure (Figure 1a), as well as isolates ISO 1S and ISO 11S that were not subjected to the scraping process (Figure 1b).

For the isolates ISO 4S, ISO 2S, ISO 11S, and ISO 3S, which underwent the scraping process, the production of conidia over time in response to UV-C radiation exposure fit a quadratic model, with the highest production occurring at 1.0 min for the first three isolates and between 1.0 and 1.5 min for the last isolate (Figure 1a and Table 1). The same quadratic model accounted for the conidium production in the ISO 2S, ISO 4S, and ISO 3S isolates, with the first two exhibiting higher production at 1.0 and 1.5 min of UV-C radiation exposure, whereas the latter reached peak production from 0 to 1.5 minutes (Figure 1b and Table 1).

The production of conidia over time in response to UV-C radiation exposure for the isolates ISO 1C, ISO 2C, and ISO 3C ultimately conformed to the Bragg model (a reparameterization of the probability density function from a Gaussian model), regardless of whether there was mycelium scraping for the first isolate, and only in the absence of scraping for the last two isolates. Taking into account the mentioned model, the highest conidia production for the ISO 1C and ISO 2C isolates occurred with 1 min of UV-C radiation exposure; meanwhile, the time of UV-C exposure that yielded the greatest number of spores for the ISO 3C isolate ranged from 1.0 to 1.5 min (Figure 1 and Table 1).

A linear and inversely proportional relationship was observed for the isolate ISO 1S, with higher conidium production when not exposed to UV-C radiation, and a reduction in the production as the exposure time increased (Figure 1a and Table 1).

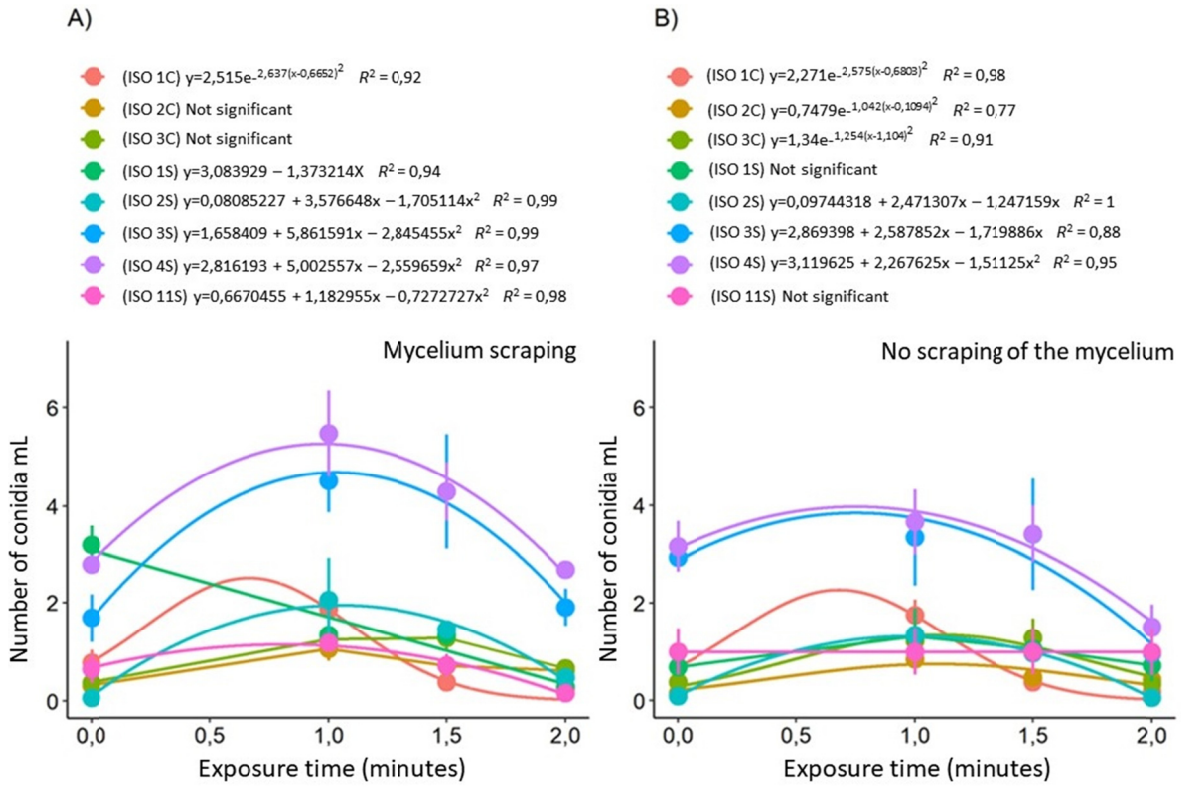

Figure 1. Mathematical models fitted for the production of *Corynespora cassiicola* conidia as a function of UV-C radiation exposure time, for eight isolates with (A) and without (B) the mycelium scraping process. The error bars represent the standard deviation from the mean (n = 5)

It was observed that, for each duration of UV-C radiation exposure, whether scraped or not, there was an increase in conidial production observed in the ISO 3S isolates (ranging from 0.97 to 4.50 conidia per mL) and ISO 4S isolates (ranging from 1.50 to 5.47 conidia per mL), compared to that of the other isolates tested. ISO 1S (3.19 conidia per mL) was an exception, as it showed no difference in conidial production without radiation exposure but with scraping. Likewise, ISO 11S (1.0 conidia per mL) showed no difference when the mycelium was not scraped yet exposed to radiation for 2 min, as compared to each mentioned exposure time (Table 1).

When subjected to the mycelium scraping process, a lower conidia count was observed in the absence of UV-C radiation exposure for the ISO 2C (0.31 conidia per mL) and ISO 2S (0.06 conidia per mL) isolates. A similar outcome was noted for the second isolate when exposed to the radiation for 2.0 min (0.47 conidia per mL), which did not differ under this condition from the ISO 1C, ISO 1S, and ISO 11S isolates and produced 0.19, 0.28, and 0.16 conidia per mL, respectively (Table 1).

When not subjected to the mycelium scraping process, it was observed that there was a lower conidia production for the isolates ISO 2C (0.84 conidia per mL), ISO 3C (1.22 conidia per mL), and ISO 11S (1.0 conidia per mL) when exposed to UV-C radiation for 1.0 min, as well as for the isolate ISO 2S (0.06 conidia per mL) when exposed to UV-C radiation for 2 min (Table 1).

Table 1. Production of *Corynespora cassiicola* conidia (per mL) in eight isolates (ISO), subjected to different UV-C radiation exposure times, with and without the mycelium scraping process

Treatment Mode	Isolates	UV-C radiation exposure duration (min)			
		0.0	1.0	1.5	2.0
With mycelium scraping	ISO 1C	0.78 BC ab[1]	1.88 B a	0.38 B b	0.19 C b
	ISO 2C	0.31 C a	1.06 B a	0.71 B a	0.61 BC a
	ISO 3C	0.38 BC a	1.25 B a	1.28 B a	0.66 BC a
	ISO 1S	3.19 A a	1.34 B b	1.34 B b	0.28 C c
	ISO 2S	0.06 C c	2.06 B a	1.46 B ab	0.47 C bc
	ISO 3S	1.68 AB b	4.50 A a	4.28 A a	1.91 A ab
	ISO 4S	2.78 A b	5.47 A a	4.28 A ab	2.69 A b
	ISO 11S	0.66 BC ab	1.19 B a	0.72 B ab	0.16 C b
Without mycelium scraping	ISO 1C	0.69 B ab[1]	1.75 B a	0.38 B b	0.19 BC b
	ISO 2C	0.16 B b	0.84 C a	0.47 B ab	0.44 ABC ab
	ISO 3C	0.38 B ab	1.22 C a	1.28 B a	0.34 BC b
	ISO 1S	0.69 B a	1.31 BC a	1.06 B a	0.72 ABC a
	ISO 2S	0.09 B b	1.34 BC a	0.97 B a	0.06 C b
	ISO 3S	2.94 A a	3.34 A a	3.40 A a	0.97 AB b
	ISO 4S	3.16 A ab	3.66 A a	3.41 A a	1.50 A b
	ISO 11S	1.00 B a	1.00 C a	1.00 B a	1.00 AB a

Note. [1] Within each treatment mode, means followed by the same letter, uppercase in the column and lowercase in the row, do not differ significantly according to the Tukey's test ($p < 0.05$).

It has been found that the scraping process can, in some instances, impact the production of conidia in *C. cassiicola* isolates. In the absence of exposure to UV-C radiation, the mycelium scraping process resulted in higher conidium production (3.19 conidia per mL) for the isolate ISO 1S compared to when scraping was not performed (0.69 conidia per mL). However, the opposite result was observed for the isolate ISO 3S, as conidium production was hindered by the scraping process (1.68 conidia per mL) compared to when scraping was not performed (2.94 conidia per mL) (Figure 2a).

It was also observed that, when exposed for 2.0 min to UV-C radiation, the scraping process resulted in a higher conidium production for the isolates ISO 2S (0.47 conidia per mL), ISO 3S (1.91 conidia per mL), and ISO 4S (2.69 conidia per mL). In the absence of scraping, conidium production for these isolates were 0.06, 0.97, and 1.50 conidia per mL, respectively. Even with the same UV-C radiation exposure time, the opposite result was noted for the isolate ISO 11S, which showed higher conidium production without scraping (1.0 conidia per mL) than that with the scraping process (0.16 conidia per mL) (Figure 2d).

Regardless of the isolates assessed after being exposed to UV-C radiation for 1.0 and 1.5 min, the scraping process did not lead to differences in conidial production; however, there seemed to be a higher conidial output for the isolates ISO 2S, ISO 3S, and ISO 4S, which was facilitated by the mycelial scraping process (Figure 2b and c).

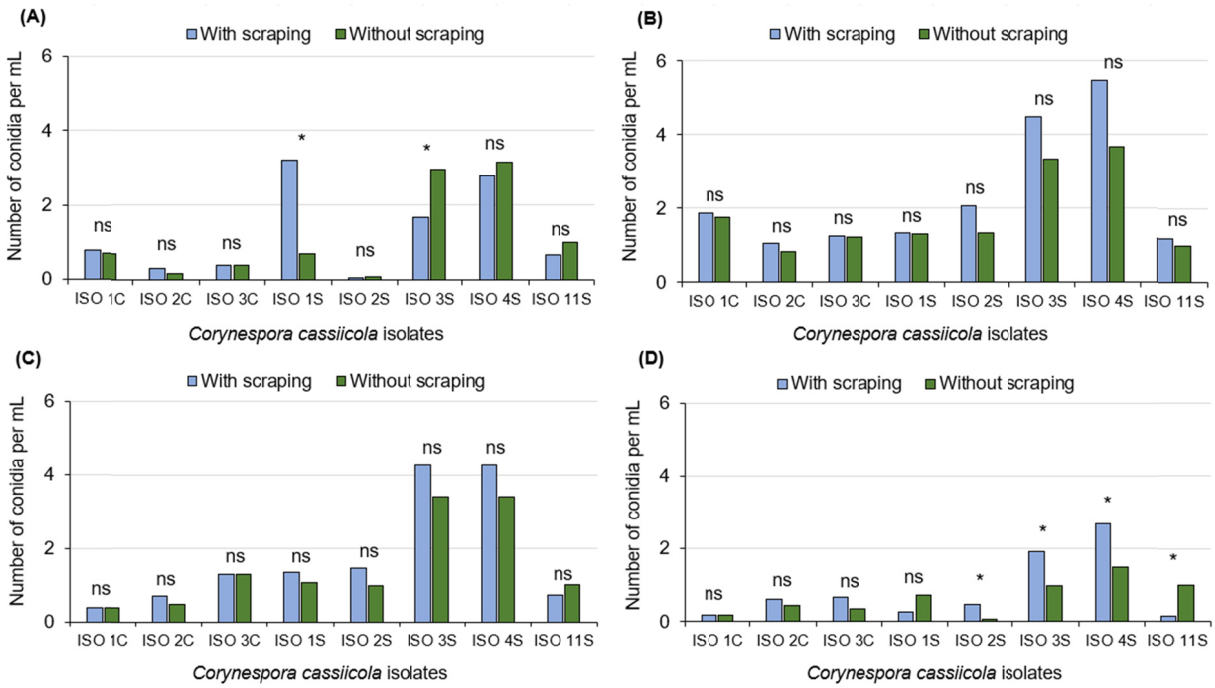

Figure 2. Conidial production (per mL) from eight isolates (ISO) of *Corynespora cassiicola* with (blue columns) and without (green columns) mycelium scraping, in the absence of UV-C radiation (a) and exposed to radiation for 1.0 min (b), 1.5 min (c), and 2.0 min (d). The abbreviation n.s. denotes not significant, while the asterisk (*) symbol signifies significant differences calculated using the Fischer's F test at a 5% significance level, between scraping and no scraping within the same isolate

4. Discussion

Light has little influence on the somatic growth of hyphae; however, it can drastically affect sporulation and the occurrence of other differentiating events in fungi (Deacon, 1996). Among all spore-activating agents, radiation has the most pronounced effect. The protocols developed to stimulate sporulation in *Stemphylium solani* employ either white or near-ultraviolet light to promote conidiophore formation, followed by periods of darkness for spore development (De Souza et al., 2018).

Continuous lighting activates key enzymes that can trigger spore production in various fungi (Trione & Leach, 1969). For species belonging to *Alternaria*, the shift from the mycelial phase to the reproductive stage requires stress factors on the colony to trigger sporulation. This induction in conidium production can be achieved by mycelial scraping followed by exposure to near ultraviolet radiation (UV-A) or continuous white light (Leach, 1967). A similar outcome was noted in our study, wherein most isolates exhibited increased sporulation when exposed to UV-C radiation for 1.0-1.5 min.

It is essential to take into account not just the chosen light spectrum but also the duration of its exposure. Excessive UV radiation, if it does not induce cell death or permanent genetic changes, can lead to metabolic alterations, such as changes in enzyme activity, membrane permeability, ion transport, or phosphate metabolism (Pulz, 2007). This type of radiation can impact biochemical processes and temporarily or permanently deactivate fungal activity, significantly influencing spore formation (Pulz, 2009).

In this study, exposure to UV-C radiation for 2.0 min decreased conidia production in most of the isolates, whether they underwent the mycelial scraping process or not. This conclusion corroborates with the findings of Braga et al. (2002) and Costa et al. (2012), where 2.0 min exposure to UV light reduced the germination of conidia from *Metarhizium anisopliae*, *Lecanicillium lecanii*, and *Clonostachys rosea*. Detrimental effects of UV radiation exposure were also noted by Oliveira et al. (2019), who found a reduction in the germination of conidia from *Trichoderma* spp. isolates after being subjected to 2.0 min of UV radiation.

According to Valle and Azevedo (1985), any ultraviolet light stimulus increases conidium production; however, to achieve maximum yield, it is essential to previously identify the type of light that is most beneficial based on the specific pathogen, as well as the culture medium used.

While light does affect conidial production, this effect is not consistent across all fungal species, nor is it observed in every isolate of the same species (Rodríguez-Romero & Corrochano, 2006, Purschwitz et al., 2008; Mello et al., 2018). Moreover, the interference of light can change depending on the chosen light spectrum; the fungus *Isaria fumosorosea* produces more conidia under white and blue light, while the opposite occurs under green and red light (Sánchez-Murillo et al. 2004).

There is a high level of genetic diversity within the species *C. cassiicola* (Dixon et al., 2009), which may explain the observed differences in conidium production induction in our study. Specifically, the ISO 1C and ISO 2C isolates with mycelium scraping and the ISO 1S and ISO 11S isolates without mycelium scraping, did not exhibit increased conidium production when exposed to UV-C radiation. In fact, there was even a decrease in conidium production under UV-C radiation exposure (ISO 1S).

The variation in the induction of sporogenesis in *C. cassiicola*, as reported by Beckman and Payne (1983), highlights the necessity to investigate the duration of exposure and the specific light spectrum required to stimulate the production of reproductive structures in fungi.

The impact of mycelium scraping on spore production in the isolates could not be determined in our study; however, significant variation has been reported in the literature regarding the stress methods used and spore production in various phytopathogenic fungi. Pulz (2007) reported that scraping aerial mycelium enhanced the sporulation of *Alternaria*. According to Rotem (1977), abundant mycelial growth inhibits fungal sporulation.

Zhu et al. (2014) noted that spore production of *Monilinia fructigena*, *Monilia polystroma*, and *Monilia yunnanensis* doubled when the mycelium underwent superficial injury through scraping, compared to when scraping was not performed.

The mycelial scraping method led to a higher spore production compared to the non-scraping approach, regardless of the *Calonectria pteridis* isolate tested. Without the initial physical injury or stress, spore formation was not stimulated (Alfenas et al., 2013). It was not possible to observe an increased conidial production in the isolates that underwent mycelial scraping, with the exception of isolate ISO 1S, which showed a significant increase in conidia following the scraping process. Similar findings were reported by Mello et al. (2018), who evaluated 28 isolates of *C. cassiicola* and noted that physical injury without light treatment did not stimulate sporulation.

This study introduces a methodology to achieve increased conidium production from *C. cassiicola* isolates, thereby enhancing the procurement of inoculum that can be used in selecting host plant lineages less susceptible to disease.

Generally, the ISO 3S and ISO 4S isolates produce more conidia than the other isolates, regardless of the scraping process or exposure to UV-C radiation. Although conidium production was not found to be affected by UV-C radiation exposure in some isolates, exposure to UV-C radiation for 1.0 min for the majority of isolates resulted in a higher conidium yield.

5. Conclusion

Generally speaking, exposure to UV-C radiation is more effective than the mycelial scraping process in the production of *C. cassiicola* conidia. The only exception is for the ISO 1S isolate, where higher conidium production was observed in the absence of UV-C radiation exposure and with mycelium scraping than in the opposite setting.

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Dra. Jacqueline D. Puia, Dr. Adriano T. Hoshino, and Dr. Marcelo G. Canteri were responsible for the study design and revising the manuscript. Leandro C. Borsato, Ana Maria Da S. Moreira, Emily D. De Almeida, Dra. Sandra C. Vigo, and Dr. Marcelo also contributed to the revision. All authors read and approved the final manuscript.

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