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# Survey of cucumber target spot, in vitro sporulation and aggressiveness of *Corynespora cassiicola*

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**Abstract**

The objective was to carry out a survey of the occurrence of the target spot (*Corynespora cassiicola*) in cucumber crops in São Paulo State; to evaluate culture media for sporulation of the pathogen and the aggressiveness of isolates of the pathogen in cucumber plants. The target spot was found in nine municipalities, being the main disease in six of the ten municipalities sampled, with leaf incidence above 50%, showing that the target spot of cucumber is widely distributed in São Paulo State. Other diseases found in lower incidences were scab (*Cladosporium cucumerinum*), alternaria spot (*Alternaria cucumerina*), cercospora spot (*Cercospora citrullina*) and downy mildew (*Pseudoperonospora cubensis*), but also present in most sampled municipalities. The zoned spot (*Leandria momordicae*) was found in samples from three municipalities and with an incidence lower than 20%. Greater sporulation of the pathogen occurred in tomato juice and oat flour media, without scraping the surface of the colony maintained for 16 days at 25ºC, under continuous fluorescent light. The germination of *C. cassiicola* isolates used in the aggressiveness test was between 82.8 and 95.5%, with the 50 isolates separated into two groups. The isolates were separated into four groups within the range of 3.1 to 22.3% in disease severity, after ten days of inoculation of the pathogen, showing the genetic variability within the species, which should be considered in management studies, such as genetic improvement.

**Keywords:** *Cucumis sativus*, culture media, fungal leaf spot, severity

## Introduction

The cucumber (*Cucumis sativus* L.) stands out among the main vegetables in Brazil, being predominantly cultivated in an agricultural greenhouse. Despite the optimization of the production system in protected cultivation, the plants cultivated in this way are still vulnerable to the occurrence of diseases, such as the target spot, caused by *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei. Epidemics of the disease have been reported in the states of Amazonas (Bezerra & Bentes, 2015), Goiás (Teramoto et al., 2011), Paraná (Verzignassi et al., 2003) and São Paulo (Fischer et al., 2021). Yield losses greater than 20% were attributed to the disease (Fischer et al., 2021); however, there is no accurate survey of the prevalence of this disease in cucumber.

The symptoms of the target spot appear as angular spots with a light brown centre and small yellow halo, and may be confused with those caused by net spot (*Leandria momordicae* Rangel), downy mildew (*Pseudoperonospora cubensis* Berkeley & Curtis) or alternaria spot (*Alternaria cucumerina* Ellis & Everh., Elliott). The coalescence of the spots causes leaf dryness, with consequent defoliation.

The fungus infects more than 500 species of plants, including mono and dicotyledons (Sumabat et al., 2018). However, the evaluation of cucumbers to *C. cassiicola* isolates from different hosts showed greater aggressiveness and shorter incubation period of isolates originating from cucumber, suggesting greater adaptation to the host species (Oliveira et al., 2006). High genetic variability among *C. cassiicola* isolates has already been reported, with the emergence of more aggressive populations in a certain location (Ferreira & Bentes, 2017).

Epidemiological studies, genotype assessment and monitoring of fungus resistance to fungicides require


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the production of conidia for bioassays. However, the low sporulation of *C. cassiicola* makes mass production of inoculum laborious (Mello et al., 2018). There is no consensus on the most favorable culture medium for pathogen sporulation. Several studies use or advocate the potato-dextrose-agar (PDA) medium in the production of inoculum (Fernando et al., 2012; Mesquini, 2012; Teramoto et al., 2013; Teramoto et al., 2017; Mello et al., 2018), with reports of other culture media considered also favorable, such as V-8 juice (Dixon et al., 2009; Parada et al. 2012), Czapek-agar (Melo & Reis, 2010, Mesquini, 2012), potato-sucrose-agar (Sousa & Bentes, 2014), carrot-pea-dextrose-agar leaves (Fortunato et al., 2015) and tomato juice (Fischer et al., 2021).

Given the lack of information about the disease in cucumber, which is important to define management strategies and allow the economic viability of the crop, this study aimed to survey the occurrence of the target spot in cucumber in agricultural greenhouses in the São Paulo State, to evaluate culture media for pathogen sporulation and to evaluate the aggressiveness of *C. cassiicola* isolates.

**Material and Methods**

*Detection of causal agent*

Cucumber leaves with symptoms of necrotic spots were collected from different cucumber producers in São Paulo State, Brazil, from August to October 2018. Each sample consisted of at least 10 diseased leaves, randomly collected from plants aged between 50 and 120 days after transplantation in agricultural greenhouses. The samples were identified according to the municipality of origin and cvs. of graft and rootstock (Table 1). The fungicides used in the cucumber crop were listed by the farmers. The leaves were stored in plastic bags and sent to the laboratory, where they were kept at 7°C until analysis. The target spot, as well as other diseases incident on the leaves, were identified through the observation of the pathogens' reproductive structures, with the aid of a stereoscopic and optical microscope (Barnett & Hunter, 2006).

In the absence of spores in the lesions, the leaves were disinfected with ethanol 70% (v/v) for one minute, followed by transfer to an aqueous solution of 1% sodium hypochlorite for one minute. Subsequently, the leaves were washed with autoclaved distilled water to remove excess hypochlorite and incubated in a humid chamber at 25ºC and 12 h photoperiod to induce sporulation in the lesions. Fragments of these diseased leaves, which were disinfected, were plated on agar-water (AA) and PDA culture medium. After four to five days, the reproductive structures of the pathogen formed on the leaves or colonies on the culture media were identified by their morphology, under an optical microscope (Barnett & Hunter, 2006). The frequency of different leaf diseases in each municipality was calculated by counting the leaves of each sample with pathogen structures and expressed as a percentage. The comparison of the incidence of diseases in the municipalities was performed through non-parametric analysis and multiple proportions comparison test, at a 5% probability level (Zar, 1999).

*Culture media for sporulation of Corynespora cassiicola*

Four *C. cassiicola* isolates from different municipalities (Arealva, Duartina, Iacanga and Ubirajara-SP) were obtained by direct isolation in PDA medium, from the pathogen's reproductive structures produced on the surface of cucumber leaves. Colony sporulation was evaluated in Petri dishes (9 mm in diameter) kept at 25ºC in BOD-type acclimatized chambers, under continuous fluorescent light, with the media PDA (Kasvi®), oat flour (OF) (14 g of agar, 40 g of oatmeal and 1000 ml of distilled water) and tomato juice (TJ) (4.5 g of $CaCO_3$, 15 g of agar, 200 ml of commercial tomato juice and 800 ml of distilled water) with or without scraping the surface of colonies grown on the culture medium. Scraping was performed with a glass slide previously flamed, in ten-day-old colonies. Petri dishes remained incubated for additional 6 days under the same conditions. The colonies were scraped with a Drigalski loop in the presence of 20 ml of distilled water and the spore concentration was evaluated using a Neubauer chamber.

The experimental design was completely randomized, in a 4 x 3 x 2 factorial scheme (isolated x culture media x with or without surface scraping of the colonies) and four replications, with each experimental unit consisting of a Petri dish. Data were expressed as number of spores per $cm^2$ of culture medium, transformed into square root and subjected to analysis of variance, with treatment means compared by Tukey test at 5% significance.

*Aggressiveness of Corynespora cassiicola isolates on cucumber*

Fifty isolates, being five isolates from ten different municipalities in São Paulo State, were obtained from samples collected during the survey carried out for the detection of cucumber foliar pathogens, described above. Isolates of *C. cassiicola* were obtained by direct isolation in PDA medium, from the reproductive structures of the pathogen produced on the surface of cucumber leaves. The multiplication of *C. cassiicola* isolates was

performed in Petri dishes with TJ medium, incubated for 16 days at 25ºC in B.O.D. climatized chambers, under continuous fluorescent light. The suspension of conidia in distilled water was adjusted to a concentration of $10^4$ conidia/ml, using a Neubauer chamber. The spore suspension (100 µl/plate) was distributed over the surface of the AA using a Drigalski loop. Conidia viability was estimated by evaluating germination 12 hours after plating in AA culture medium. Conidia with the length of the germ tube equal to or greater than the length of the conidia were considered germinated (Teramoto et al., 2013). One hundred conidia per plate were evaluated, out of a total of three plates per isolate of the pathogen, in a completely cazualized design.

Cucumber plants cv. Soldier were grown in plastic pots (3 l) containing commercial pine-based substrate (Carolina Soil Standard®). Direct sowing of two seeds per pot was carried out, with the thinning of one seedling in case of emergence of two seedlings.

Plant inoculation was carried out by spraying the suspension of conidia on the second and third definitive leaves, completely expanded, on both sides, up to the runoff point, 30 days after sowing. Then, the aerial part of the plants was covered with a transparent plastic bag moistened for 24 hours, aiming at the formation of a humid chamber, returning soon after to the condition of a greenhouse.

The disease severity (% of the affected leaf area) in the two inoculated leaves was estimated visually with the aid of a diagrammatic scale with seven levels of severity (0.3; 0.8; 2; 5; 11.5; 25 and 46 %) (Teramoto et al., 2011), five and ten days after pathogen inoculation. To verify the formation of spores on the inoculated leaves, adhesive tapes were lightly pressed over the lesions on the abaxial surface of the leaf and transferred to optical microscope slides. At least five lesions per plant were sampled, including the largest lesions on a leaf. The reaction was considered positive (susceptible) due to the presence of disease symptoms and signs of the pathogen in leaf limbs (Oliveira et al., 2006). The experimental design used was completely randomized blocks, with 50 treatments (isolates) and four replications, each experimental unit consisting of a plant. The median and variation in disease severity obtained with the isolates from each municipality, at 5 and 10 days after inoculation, were calculated and expressed in a box plot graph.

The mean results of pathogen germination and disease severity were transformed into arcsine of the root of the proportion (X= arcsine √%) and subjected to analysis of variance and treatment means were compared by the Scott-Knott test at 5% of significance. The experiments were repeated once and the data analyzed together, due to the low variability of the variables analyzed between the experiments. Aiming to analyze a possible relationship between % germination and disease severity, 10 days after inoculation, a Pearson correlation analysis was performed.

**Results and Discussion**

*Detection of causal agent*

Twenty-eight samples of cucumber leaves with symptoms of necrotic spots were collected for the diagnosis of incident diseases, coming from 24 producers in 10 different municipalities (Table 1). During the samplings, cucumber producers reported using the following fungicides (active ingredient) for disease management in cucumber plants: azoxystrobin, difenoconazole, iprodione, mancozeb, copper oxychloride, pyraclostrobin, tebuconazole, thiophanate-methyl, azoxystrobin + difenoconazole, cymoxanil + mancozeb, metalaxyl-M + chlorothalonil, methyram + pyraclostrobin, pyraclostrobin + fluxapyroxad,thiophanate-methyl + chlorothalonil and trifloxystrobin + tebuconazole, of which only iprodione and cymoxanil + mancozeb are not registered in the Brazil for the cultivation of cucumber (Agrofit, 2022), employed in four and nine agricultural greenhouses, respectively. However, although the mixture cymoxanil + mancozeb is not registered for cucumber, the active ingredient cymoxanil is registered in the form of the mixtures cymoxani + zoxamide and cymoxanil + famaxadone, recommended for the control of downy mildew.

The target spot was found in nine municipalities, being the main disease in the samples from six of the ten municipalities, with a foliar incidence above 50%. The disease was the most frequent in the average of the ten municipalities, with 49.4% of incidence, showing that the target spot of cucumber is widely distributed in the State of São Paulo (Table 2). Other diseases found in lower incidences were scab (*Cladosporium cucumerinum Ellis* & Arthur), alternaria spot, cercospora spot (*Cercospora citrullina* Cooke) and downy mildew, but also present in most of the sampled municipalities (Figure 1, Table 2). The net spot was found in samples from three municipalities and with an incidence of less than 20%.

In a leaf sample from São Pedro do Turvo, no fungal or bacterial pathogens were detected even after a humid chamber and isolation in a culture medium, possibly due to some phytotoxicity, since, according to a report by the rural producer, there was no increase in the occurrence of spots in the days following sampling. It is noteworthy that the symptoms of the diseases found are similar (Figure 1) and that their identification was only

possible after observation of the reproductive structures of the pathogens under a stereoscopic and/or optical microscope.

**Table 1.** Information on the survey of cucumber foliar fungal diseases carried out in agricultural greenhouses in São Paulo State, between august and october 2018.

| Sampling/ farm * | Municipality | Cultivar/rootstock | Number of leaves |
|---|---|---|---|
| 5/5 | Arealva | Soldier, Valent, Kobayashi/Keeper, Soldier/Keeper | 81 |
| 1/1 | Avaí | Soldier/Potent | 17 |
| 2/1 | Bariri | Safira, Tsuyataro | 21 |
| 1/1 | Duartina | Valent | 18 |
| 1/1 | Fernão | Valent/Potent | 10 |
| 3/2 | Guarantã | Soldier/Potent | 54 |
| 4/4 | Iacanga | Tsuyataro, Soldier/Keeper, Taiko/Potent | 62 |
| 5/3 | Reginópolis | Valent, Natsubayashi/Keeper, Natsubayashi/Potent | 85 |
| 2/2 | São Pedro do Turvo | Valent/Potent, Soldier/Potent | 23 |
| 4/4 | Ubirajara | Soldier/Keeper, Valent/Keeper | 70 |

* Number of samples with at least 10 symptomatic cucumber leaves/number of farms from which the samples were collected in each municipality

**Table 2.** Incidence of fungal pathogens on cucumber leaves with leaf spots, collected in agricultural greenhouses in São Paulo State, between august and october 2018.

| Municipality | Average incidence (minimum and maximum) of pathogens (% of leaves with symptoms) | | | | | |
|---|---|---|---|---|---|---|
| | *Corynespora* | *Cladosporium* | *Alternaria* | *Cercospora* | *Pseudoperonospora* | *Leandria* |
| Arealva | 27.2 b (0-100) | 54.3 a (23.1-100) | 24.7 b (0-72.2) | 13.6 b (0-50) | 16.0 b (0-78.6) | 0.0 c* |
| Avaí ** | 100.0 a | 5.9 b | 5.9 b | 0.0 b | 0.0 b | 0.0 b |
| Bariri | 0.0 c | 28.6 b (20.0-36.4) | 85.7 a (81.8-90.0) | 9.5 bc (9.1-10.0) | 0.0 c | 19.0 bc (10.0-27.3) |
| Duartina ** | 88.9 a | 0.0 b | 0.0 b | 0.0 b | 11.1 b | 0.0 b |
| Fernão ** | 80.0 ab | 20.0 bc | 0.0 c | 90.0 a | 0.0 c | 0.0 c |
| Guarantã | 100.0 a | 0.0 c | 11.1 b (5.6-19.2) | 11.1 b (0-23.1) | 3.7 bc (0-11.1) | 0.0 c |
| Iacanga | 54.8 a (0-100.0) | 16.1 b (0-46.2) | 9.7 b (0-35.3) | 16.1 b (0-100.0) | 0.0 c | 14.5 b (0-69.2) |
| Reginópolis | 14.1 b (0-31.6) | 41.2 a (14.3-93.3) | 17.6 b (0-36.8) | 25.9 ab (0-77.8) | 32.9 ab (0-89.5) | 15.3 b (0-92.9) |
| São Pedro do Turvo | 56.5 a (0-100.0) | 0.0 b | 0.0 b | 0.0 b | 13.0 b (0-23.1) | 0.0 b |
| Ubirajara | 60.0 a (0-100.0) | 24.3 b (0-69.6) | 11.4 bc (0-34.8) | 5.7 cd (0-17.4) | 21.4 bc (0-100.0) | 0.0 d |
| Total | 49.4 a | 26.1 b | 16.8 c | 14.5 c | 14.3 c | 5.9 d |

*Data followed by the same lowercase letter on the line do not differ from each other by non-parametric analysis and multiple proportion comparison test, at 5% probability level (Zar, 1999).

** In these municipalities, sampling was carried out in a single agricultural greenhouse.

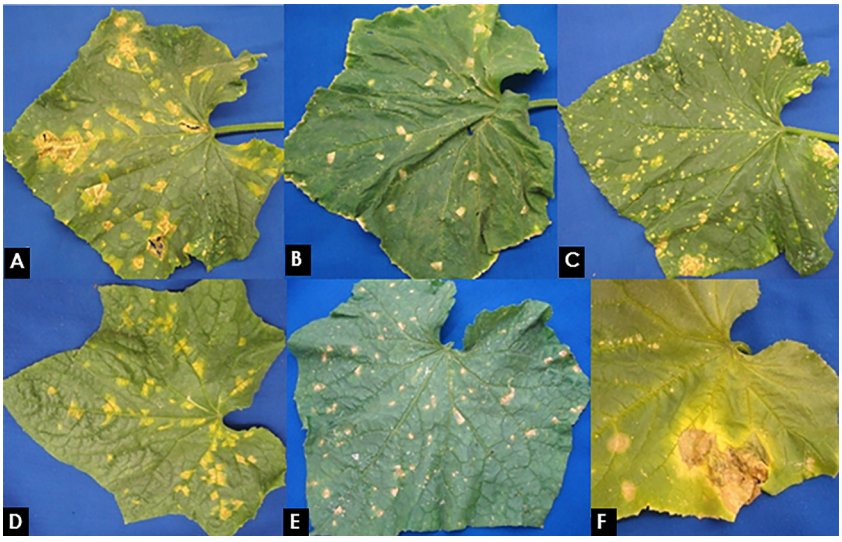


**Figure 1.** Symptomatology of cucumber leaf diseases observed in surveys carried out in different municipalities of São Paulo State. Cucumber leaves with symptoms of alternaria spot (A), cercospora spot (B), scab (C), downy mildew (D) and target spot (E, F).

The target spot was found in Natsubayashi, Soldier and Valent cvs., despite the first being classified as moderately resistant and the others highly resistant (Takii, 2021). In evaluating the response of ten cvs. of cucumber to the target spot, multivariate analysis with the epidemiological components combined identified three groups of cultivars, with Soldier allocated in the most susceptible group, Valent in the intermediate group and Safira in the least susceptible group (Fischer et al., 2021). It is noteworthy that in sampling with Safira cv., in a property located in the municipality of Bariri-SP, the target spot was not detected.

Plants are considered sick when they present abnormal development, expressed by visible symptoms that compromise the quality and/or the economic value of the crop (Zambolim et al., 2012). Yield losses of up to 60% and reduction in fruit quality have already been attributed to the target spot in cucumber, in the Paraná State (Verzignassi et al., 2003). Not only in cucumber, but in other crops, this disease has been reported to cause serious damage, such as in soybeans (Molina et al., 2018) and cotton (Fulmer et al., 2012). In a survey of the soybean target spot in 43 leaf samples from the states of Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul, Rondônia and São Paulo, in the period 2009 and 2010, the disease was present in 88% of the samples. The genera *Cercospora*, *Colletotrichim*, *Corynespora* and *Fusarium* were identified in the samples, with a predominance of *C. cassiicola* (Avozani, 2011). The correct identification of pathogens, through diagnostic processes, is necessary in order to reduce costs with pesticides and inputs in general. The most modern fungicides, with increasingly specific active ingredients, act differently in each fungal species. While effectively controlling one species, they can be completely ineffective for another. Therefore, diagnosis and control are closely related, as diagnosis determines control.

*Culture media for Corynespora cassiicola sporulation*

Culture media, pathogen isolates and colony scraping, as well as the interaction of these treatments, showed significant differences (Table 3). It was observed that the scraping of the culture media after 10 days of colony growth compromised the sporulation of the isolates in TJ and OF media and that differences in sporulation between the four isolates of the pathogen were observed in the three media evaluated, regardless of the superficial scraping of the colonies. In general, TJ and OF media, without superficial colony scraping, were the most favorable to pathogen sporulation, with Duartina isolate showing higher sporulation on OF medium and Arealva and Iacanga isolates showing higher sporulation on TJ medium. One of the characteristics that contributes to the choice of TJ medium in relation to OF medium is the lower production of mycelial mass in TJ medium, facilitating the removal of spores from the surface of the culture medium by filtering the spore suspension.

**Table 3.** Sporulation of *Corynespora cassiicola* ($10^4$ conidia/$cm^2$) isolates from cucumber at 16 days after incubation at 25°C in potato-dextrose-agar (PDA), oat flour (OF) and tomato juice (TJ) culture media, with and without surface scraping of the colonies performed on the tenth day.

| *C. cassiicola* isolates | Culture media[1] | | |
|---|---|---|---|
| (municipalities) | PDA | OF | TJ |
| | With surface scraping of the colonies | | |
| Arealva | 0.26 aA | 0.28 aA | 3.02 bB* |
| Duartina | 0.43 aAB | 3.97 cB | 1.42 bA |
| Iacanga | 0.99 aB | 5.88 bB | 1.76 aAB |
| Ubirajara | 1.14 aB | 5.44 bB | 1.82 aAB |
| Average | 0.71 aa | 3.89 ba | 2.01 aba |
| | Without surface scraping of the colonies | | |
| Arealva | 0.21 aA | 3.50 bA | 10.68 cB |
| Duartina | 0.73 aAB | 13.39 cC | 7.89 bA |
| Iacanga | 0.19 aA | 8.16 bB | 12.65 cB |
| Ubirajara | 0.92 aB | 13.20 bC | 12.07 bB |
| Average | 0.51 aa | 9.56 bβ | 10.82 bβ |
| CV (%) | | 8.68 | |

*Data followed by the same letter, lowercase in the row and uppercase and greek in the column, do not differ at a level of 5% by Tukey's test. Statistical analysis was performed after transforming the data into square root.

In evaluation of conidia production of three isolates of *C. cassiicola* from rubber trees on PDA, Lima bean-agar, Czapek-Dox-agar, malt-agar, corn flour-agar, agar-agar and rubber leaf extract media -agar, differences were observed between isolates, with greater sporulation in PDA medium for two isolates (19.3-216.7 x $10^4$ spores/$cm^2$) and absence of sporulation in culture media evaluated for one isolate (Fernando et al., 2012). In another study with *C. cassiicola* from rubber trees, Fernando et al. (2011) had already found variability between isolates for the in vitro production of conidia, with the isolates being classified as high, moderate and low sporulation.

As for *C. cassiicola* isolates from soybean, the Czapek-agar solution medium promoted greater sporulation compared to PDA, infant food, malt agar, OF and V8 agar juice medium, in a 12-hour photoperiod and overlaying filter paper on the substrate (Melo & Reis, 2010), and contrary to what was observed in the present work, the PDA medium provided greater sporulation compared to the OF medium, in a 12-hour photoperiod. Significant interaction between ten isolates of the soybean pathogen and two culture media was also observed by Mesquini (2012), with the PDA medium being more favorable to sporulation for three isolates and the Czapek-agar medium for two other isolates.

The ten isolates of the pathogen from soybeans showed sporulation in PDA ranging from 1.3 to 13.6 x $10^4$ spores/$cm^2$ (Mesquini, 2012), while those from cucumber showed comparatively lower sporulation in PDA (0.19-1.1 x $10^4$ spores/$cm^2$) and a comparable sporulation in TJ and OF media (3.5-13.4 x $10^4$ spores/$cm^2$) (Table 3). The data obtained in this study corroborate the results obtained in rubber tree (Fernando et al., 2012) and in soybean (Mesquini, 2012), in which the sporulation of *C. cassiicola* isolates is variable in different culture media.

Mechanical stress resulting from surface scraping of *C. cassiicola* soybean colonies showed, as in the present study, a variable response in sporulation as a function of the pathogen isolate, with a significant increase in sporulation in four isolates and a reduction in one isolate, among the 21 isolates evaluated (Mello et al., 2018). This variation in behavior between different isolates can be explained by the high genetic diversity of the *C. cassiicola* species (Dixon et al., 2009; Mello et al., 2018). Although the superficial scraping of colonies is a practice recommended by some authors to stimulate sporulation in PDA (Miyamoto et al., 2009) and V8 media (Dixon et al., 2009), in TJ and OF media, scraping impaired the sporulation of cucumber isolates and should not be adopted.

*Aggressiveness of Corynespora cassiicola isolates from cucumber plants*

Germination of *C. cassiicola* isolates was greater than 80.0% after 12 h of incubation (Figure 2), with the 50 isolates separated ($p < 0.05$) in two groups (82.8-89.0% and 89.5-95.5%). In the case of *C. cassiicola* isolates from several host species, the time period for the conidia to reach 85% germination ranged from 5.0 to 20 h (Teramoto et al., 2013). In that work, this index ranged from 7.6 to 9.0 h for *C. cassiicola* isolates from cucumber, regardless of the place of origin of the isolates. In rubber tree pathogen isolates, maximum germination (100%) was observed after 12 h of incubation, with a rapid increase in germination after 5 h (Fernando et al., 2012).

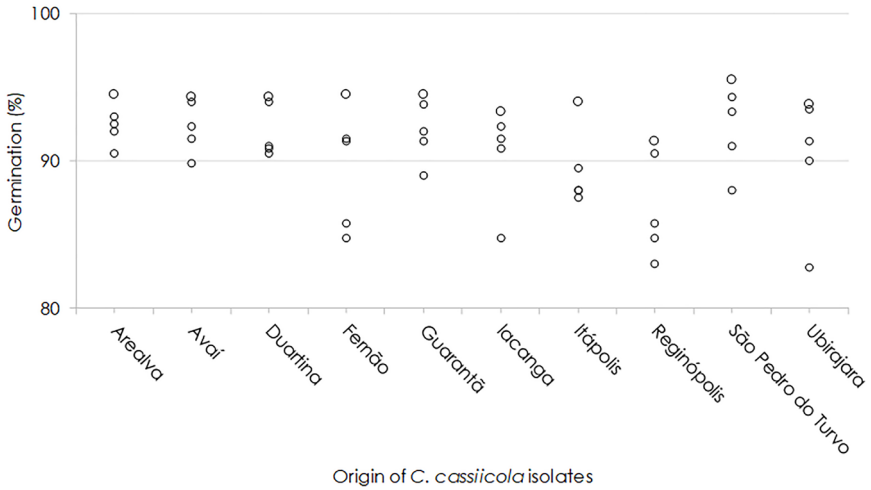


**Figure 2.** Germination (%) of conidia from 50 isolates (five isolates per municipality) of *Corynespora cassiicola* from cucumber in agar-water culture medium, 12 h after incubation at 25°C, in the dark.

All *C. cassiicola* isolates caused target spot symptoms and the severity was also variable as a function of the isolates (Figure 3), with the 50 isolates separated ($p < 0.05$) into four groups within the range of 1.1 to 10.0% of disease severity (1.1-3.2%; 3.4-4.1%; 4.2-6.2% and 6.6-10.0%), five days after the pathogen inoculation. The disease severity was between 3.1 and 22.3% 10 days after inoculation, (Figure 3), with the isolates allocated ($p < 0.05$) in four severity groups (3.1-7.5%; 7.8-13.2%; 16.1-19.0% and 20.4-22.3%). In general, there was no relationship between the severity level and the origin of the isolate, except for the Arealva isolates, with an intermediate behavior after 10 days of inoculation (8.9 to 13.2% of severity). However, highly aggressive isolates, with disease severities above 15%, were detected only in the municipalities of Avaí, Reginópolis and Itápolis (Figure 3). Differences in disease severity have already been reported between *C. cassiicola* isolates originating from different hosts. The greatest disease severity in cucumber was observed when the plants were inoculated with isolates originating from this culture (Oliveira et al., 2006). In tomato, differences in aggressiveness were also observed between isolates from different origins (Ferreira & Bentes, 2017). Differences in aggressiveness result from the genetic variability within the species, as already observed among isolates of the pathogen from different

hosts and geographic regions (Dixon et al., 2009).

Thousands of supposed genes associated with virulence have been identified in *C. cassiicola*, providing information about the pathogenic mechanism of this pathogen in cucumber (Gao et al., 2020). In other hosts, effector proteins, cassiicolin, secreted by *C. cassiicola* have already been identified. This effector contains six different cassiicolin isoforms, produced by the Cas1, Cas2, Cas3, Cas4, Cas5 and Cas6 genes, in different *C. cassiicola* isolates sampled from various hosts and geographic origins (Déon et al., 2014; Wu et al., 2018). The aggressiveness of the isolates was related to the type of cassicolin isoform, and the isolates carrying the Cas1 gene were the most aggressive to rubber trees. In addition, some isolates without the Cas gene also generated moderate symptoms in rubber tree leaves, showing that there must be other effectors not yet characterized in *C. cassiicola* (Déon et al., 2014).

In a study of the pathogenicity of *C. cassiicola* on different hosts, the isolates obtained from Iranduba were more aggressive than those from Presidente Figueiredo and Manaus-AM, suggesting the occurrence of more than one population of the pathogen in these areas (Ferreira & Bentes, 2017). Nghia et al. (2008), using Internal Simple Sequence Repeat (ISSR) analysis with 8 primers, in rubber tree pathogen isolates from different regions of Malaysia, found two distinct groups separated in relation to the geographic region of collection. However, several works using molecular techniques of Restriction Fragment Length Polymorphism (RFLP) and Random Amplification Polymorphic DNA (RAPD) (Silva et al., 1995; Romruensukharom et al., 2005), genetic sequencing (Dixon et al., 2009) and ISSR with 16 primers (Qi et al., 2011), did not find agreement between *C. cassiicola* isolates regarding their geographic location.

Sporulation was found in the lesions for 60% of the isolates, five days after inoculation, and for 96% of the isolates, 10 days after inoculation, although two isolates, one from Fernão and the other from Itápolis, sporulated only after three days in the humid chamber, in leaf samples collected ten days after inoculation. There was no significant correlation (r = -0.12) between % conidia germination and disease severity 10 days after inoculation, possibly due to the high percentage of conidia germination of all isolates.

The three isolates considered more aggressive, with 19.0; 20.4 and 22.3% of disease severity, originating, respectively, from Reginópolis, Itápolis and Avaí, were preserved in the Mário Barreto Figueiredo Micoteca, of the APTA, Biological Institute of São Paulo, with the names MMBF 01/20 , 02/20 and 03/20, respectively, and selected for studies to evaluate the behavior of cucumber genotypes in relation to the disease (Fischer et al., 2021).

**Conclusions**

The target spot was the most frequent leaf disease in cucumber in São Paulo State.

Greater sporulation of *C. cassiicola*, from cucumber, occurred in tomato juice and oat flour media, without scraping the colony surface.

Isolates of *C. cassiicola* showed low variability in spore germination, with germination above 82%; while for aggressiveness in cucumber plants, the isolates were separated into four groups, showing the genetic variability within the species, which should be considered in management studies, such as breeding programs.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.