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*Full Length Article*

# Climatic and Territorial Vulnerability in the Spatio-Temporal Occurrence of Citrus Black Spot in the Eastern Amazon

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## Abstract

Citrus black spot caused by *Phyllosticta citricarpa* Van der Aa is a quarantine pest that causes premature fruit drop, restricts exports, and results in significant losses in Brazilian citrus farming production, where its occurrence must be monitored. This study aimed to analyze the spatio-temporal dynamics and seasonal patterns of prevalence and incidence of citrus black spot in Eldorado do Carajás, Pará, Brazilian Eastern Amazon. A total of 181 properties were inspected, showing prevalence rates of 5.6, 4 and 7.8%, and annual average incidence rates of 62, 73, and 61% in 2019, 2021 and 2022, respectively. A total of 439 diseased plants were eradicated across 11 foci, with higher incidence in the drier period (81.8%) compared to the wetter period (18.2%). This was influenced by relative humidity, minimum temperature, and rainfall. The incidence was diagnosed in 90% of domestic plantings and 10% of commercial production units. The citrus species with the highest incidence were sweet orange (52.9%), tangerine (29.4%) and rangpur lime (17.6%) in orchards over 10 years old. At the property level, incidences showed an aggregated spatial pattern explained by the Gaussian model and autocorrelation *via* Moran's Index, with disease spread ranging from 25.5 to 103 m. At the municipal level, the foci were spatially random. Mapping revealed that rural properties with foci were easily accessible for the sale of contaminated seedlings, posing a threat to the competitiveness of citrus fruit marketing and the sustainable development of Amazonian citrus farming.

**Keywords:** Amazonian citriculture; Geostatistics; Geographic Information System; Phytosanitary survey; *Phyllosticta citricarpa*

## Introduction

The Brazilian citrus industry is the largest in the world, and the state of Pará ranks first in the Brazilian Amazon in production, with approximately 19.214 hectares of planted area (IBGE 2022). It is the 7$^{th}$ largest producer of oranges and the 2$^{nd}$ largest of Tahiti acid lime in Brazil (FAO 2022). However, citrus black spot, caused by the fungus *Phyllosticta citricarpa* Van der Aa, poses a significant phytosanitary threat to this production chain (Magarey *et al.* 2015).

Establishment of citrus centers in Pará has boosted the production and marketing of fresh fruit and juice by opening new markets (Agência De Defesa Agropecuária Do Pará 2017). However, citrus black spot (CBS) restricts fruit exports to countries where the quarantine pest is absent, such as those belonging to the European Union (Silva-Júnior *et al.* 2016). Phytosanitary surveys serve as official control measures, aiming to detect the presence or absence of quarantine pests in the state of Pará to ensure the health and

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competitiveness of products in both national and international markets (Agência de Defesa Agropecuária do Pará 2023).

In 2019, the pest was detected for the first time in the state of Pará, in the municipality of Eldorado do Carajás, in a commercial citrus plantation by the Pará Agricultural Defense Agency (ADEPARÁ). CBS is a quarantine pest present in Brazil since 1980, with restricted geographic distribution and under official control (Ministério da Agricultura 2018a), occurring in the northern region in the states of Amazonas in 2003 (Gasparotto and Pereira 2004) and Rondônia in 2008 (Vieira-Junior *et al*. 2010).

The fungus has two dispersal mechanisms: ascospores (sexual spores) (*Guignardia citricarpa* Kiely), which are produced in pseudothecia from decomposing citrus leaves in the soil and spread by wind, introducing the disease to new areas, and pycnidiospores (asexual spores) (*P. citricarpa*), produced in pycnidia on fruit lesions, leaves attached to plants, and dry branches, disseminated over short distances by water (Kotzé 1981).

All varieties and hybrids of sweet oranges, tangerines and true lemons are susceptible to the pest. It can cause more than 80% of premature fruit drop due to weather conditions and the presence of the pathogen with a long dissemination and incubation period (Silva-Júnior *et al*. 2016). CBS introduced by infected plant material of the *Citrus* genus causes lesions, fruit rejection by the industry, increases the cost of chemical control for rural producers and promotes socio-environmental impacts on the citrus chain (Spósito *et al*. 2011).

In this sense, research on seasonal, temporal and geographic dynamics at various scales is strategic to support official control actions, management programs for pest eradication (Byamukama *et al*. 2014) and to identify periods of susceptibility of orchards in the Amazon. In order to improve knowledge of the incidence of the pest under field conditions (Vieira-Júnior *et al*. 2012), this study aimed to quantify, map and analyze the spatiotemporal dynamics and seasonal patterns of prevalence and incidence of citrus black spot in the municipality of Eldorado do Carajás, Pará, Eastern Amazon, Brazil.

## Materials and Methods

### Phytosanitary survey for the detection of *P. citricarpa*

Phytosanitary detection surveys were carried out biannually in 2019, 2021 and 2022, in 66 municipalities of the state of Pará by the Pará Agricultural Defense Agency (ADEPARÁ) (Fig. 1A), in commercial and domestic citrus plantations with detection of *P. citricarpa* in the municipality of Eldorado do Carajás, Pará, eastern Amazon, Brazil (Fig. 1B). The municipality of Eldorado do Carajás is located in the Legal Amazon region (-6°8'30.94" S; -49°23'24.8" W; altitude of 152 m). It features dense forest vegetation on flat and rugged terrain, with latosols and argisols, 6 hectares of citrus orchards, and an average yield of 8 to 12 tons per hectare (IBGE 2022). The climate is classified according to the Köppen system as type "Am", characterized by a tropical climate with a dry season, with average annual air temperature and radiation of 27.4°C and 169 W $m^{-2}$, respectively (Alvares *et al.* 2013).

Under field conditions, ADEPARÁ technicians inspected a total of 6.753 plants of the genus *Citrus* on 181 properties through monitoring with the initial detection of CBS, 19, 52 and 110 in the years 2019, 2021 and 2022, respectively, in an area of 24.31 ha. Each rural property was inspected only once during the surveys, in order to obtain representative geographic coverage of the municipality and the presence or absence of the pest (Fig. 1C).

All plants in domestic plantations and 20% of plants in commercial plantations were inspected by systematic sampling, starting in the first planting row of the area border, inspecting one in every five plants, or one in every five planting rows, with inspection of all plants in the row (Brazil 2018b) and when a single fruit with suspicious symptoms was detected, the plant was considered diseased (Brazil 2008).

Supplementary data collected in the field included information about the producer, property, cultivated area, number of plants, number of suspected plants, *Citrus* species and related genera, geographic coordinates and planting age. A diagrammatic scale was used for symptom identification (Aguilar-Vildoso *et al.* 2002) and when there was a case of suspicion, inspections were interrupted as a precaution to prevent the spread of the plague to other properties.

The visual assessment of disease incidence involved inspecting fruits on the outer part and the lower third of the plants, where there is greater exposure to light and fruits exposed to the sun with possible symptoms of virulent, tough, pitted, cracked, lacy, and false melanosis (Fig. 2) (Brazil 2008; Silva-Júnior *et al.* 2016), whose fungal symptoms can also be expresses in leaves, petioles, branches, thorns and peduncles (Feichtenberger *et al.* 2005).

Following the official phytosanitary diagnosis report from the confirmation laboratory confirming the presence of CBS, all citrus plants in commercial, non-commercial, urban properties, and public areas adjacent to the affected property within a minimum radius of one kilometer were inspected (Brazil 2018b).

### Detection of citrus black spot

Plants with suspected symptoms of CBS were identified using the direct method, with samples consisting of 5 units of branch, leaf and fruit being collected, packaged in a brown paper bag, labeled, filled in with a pencil and in a Styrofoam cooler box, and sent to the Agronomic laboratory in Porto Alegre (RS) for phytosanitary diagnosis using molecular and biological methods (Ministério da Agricultura 2008, 2018b).

The laboratory test for induction of CBS symptoms

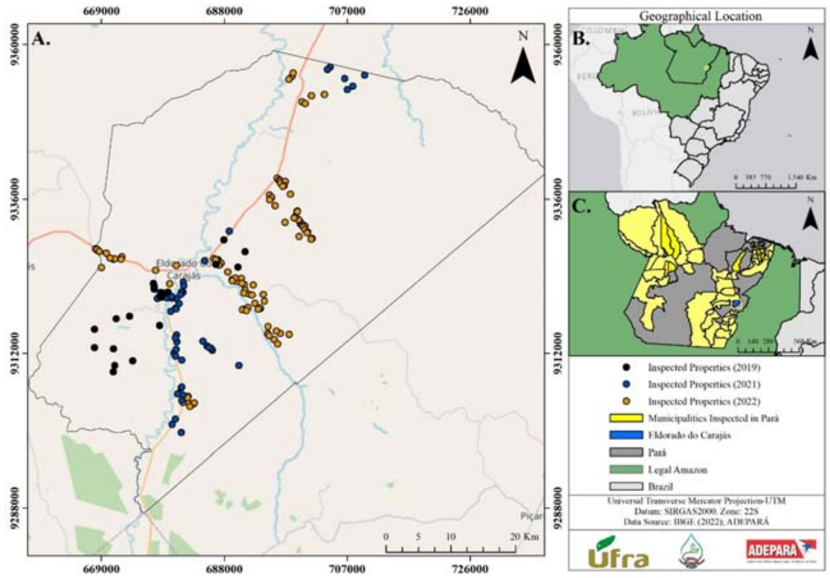


**Fig. 1:** Survey areas for detection of citrus black spot in the state of Pará (A) and monitored rural properties (B) in the municipality of Eldorado do Carajás, Pará, Brazil in 2019, 2021 and 2022 in the Amazon (C)


**Fig. 2:** Sweet orange (**A**, **B**, **C**), tangerine (**D**) and key lime (**E**, **F**) fruits inspected with symptoms of tough to virulent spots on rural properties with the presence of citrus black spot in Eldorado do Carajás, Pará, Brazil

was performed by immersing the fruits in a solution containing Ethephon at 750 ppm for five minutes, followed by incubation at temperatures above 25ºC for a minimum period of 28 days. Visual and microscopic observations were performed weekly on the incubated fruits. If symptoms occurred, the causal fungal agent was isolated by means of a culture of carrot agar supplemented with dextrose or oat agar (Ministério da Agricultura 2008).

### Data on annual prevalence and incidence of citrus black spot

Prevalence data at the municipality scale were defined as the number of citrus plantations with at least one positive sample of CBS divided by the total number of inspected properties, multiplied by 100. The incidence of CBS was calculated at the property geographic scale by dividing the number of plants with positive samples for CBS within each property by the total number of plants inspected in each sampled property with CBS (Byamukama *et al.* 2014).

### Seasonality in the prevalence and incidence of citrus black spot

The seasonality of disease prevalence each year was determined by the number of properties positive for CBS divided by the total number of inspected properties, multiplied by 100, during the wettest and driest periods. The seasonality of disease incidence each year was determined by the number of plants positive for CBS divided by the total number of inspected plants, multiplied by 100, during the wettest and driest periods (Byamukama *et al.* 2014).

The rainiest period from January to April and the least rainy period from May to December were determined by the climatological water balance method of Thornthwaite and Mather (Rolim *et al.* 1998), based on the soil characteristics of the municipality (Doorenbos and Kassam 1994; IBGE 2022) and the effective depth of the orange tree root system from 0 to 40 cm (Testezlaf *et al.* 2007), corroborated by Ferreira Filho *et al.* (2020).

Climatic data on maximum, average, minimum air and dew point temperature (ºC), wind direction and speed (m $s^{-1}$), global radiation (MJ $m^{-2}$ $day^{-1}$), precipitation (mm) and relative humidity (%) were used to analyze environmental conditions and perform the water balance, obtained by satellite information and the MERRA-2 assimilation model from 1981 to 2022 from the National Aeronautics and Space Administration/Prediction of World Wide Energy Resources (NASA/POWER https://power.larc.nasa.gov) platform with a horizontal resolution of 1° latitude-longitude

(Monteiro *et al*. 2018) because the municipality does not have a surface meteorological station available and in the WRPLOT software version 8.0.2. the compass rose was created.

**Spatial data analysis**

The incidences of CBS in the municipality were plotted to map and identify the geographic areas with the presence of the disease by collecting geographic coordinates in the field using the Global Positioning System (GPS), using the SIRGAS2000 geodetic reference system, where they were transformed *via* geographic information system (GIS) to Universal Transverse Mercator Cartographic Projection (UTM). Interpolated maps were used to describe the incidence levels on the properties estimated by the ordinary kriging method and inverse squared distance (ISD) which considers the closest points, more similar than the most distant points (Silva *et al.* 2019) using ArcGis 10.5 software. The spatial dependence of CBS incidence levels at the property and municipality scales was analyzed by semivariograms and Moran's Index (MI) ranging from -1 (weak spatial dependence) to 1 (strong spatial dependence) (Byamukama *et al*. 2014) using the geostatistical analysis procedure of the Smart-Map plugin of the QGIS 3.28.12 software.

Based on the statistical parameters of the coefficient of determination (precision) and root mean square error (accuracy) of the test, the Gaussian theoretical model presented the best fit to the empirical semivariogram of CBS incidence (Yamamoto and Landim 2013). The semivariogram expresses the spatial variability of dissimilarity between pairs of points due to the distance (h) and orientation between two sampling locations (Seidel and Oliveira 2014). Semivariance measures the degree of spatial dependence between samples, assuming stationarity in the propagation of diseased plants, where samples that are closer together tend to behave more similarly than those separated by greater distances (Nanos *et al*. 2004).

Semivariograms are theoretical models whose semivariance values increase with distance to level off at the maximum semivariance known as the threshold (h), which is the distance at which the samples present random static variability ($C_0$ + C + r). The range or amplitude (a) is the maximum distance at which one sample is related to another (spatial dependence) and there is no further increase in semivariance, defining the sampling grid in the field. The nugget effect ($C_0$) is the random semivariance on the short scale that represents the real variability in the field of the spatial phenomenon and indicates the suitability of the sampling scale to represent reality (Yamamoto and Landim 2013).

**Risk factors associated with citrus black spot**

To determine the influence of geographic position on the incidence of CBS, the geographic coordinates (latitude and longitude) of the properties (x) were plotted against the CBS incidence of each property in the municipality (y) for analysis of north-south and east-west gradients. The incidence percentages were regressed against latitude and longitude (x) to test for significant linear relationships.

The influence of climatic conditions on the incidence of CBS was assessed using the multivariate method of principal component analysis to identify similar and dissimilar groups, reduce data dimensionality with minimal information loss (Junior and Almeida 2017), and retain, in estimated order, the maximum amount of information for total variation and recognition of patterns in the overall data distribution comprehensively and realistically (Dallastra *et al.* 2014). The analyses were conducted in the Python programming language (https://www.python.org/, version 3.7.3), observing assumptions of Bartlett's test and the Kaiser-Meyer-Olkin (KMO) index.

Based on the analysis of environmental conditions and pest occurrence, the main transit risk routes in different modes present in the state of Pará were mapped, and municipalities allowing the entry of plant propagation material were identified as gateways to the main citrus-producing areas.

## Results

Through the analysis of the spatiotemporal dynamics of the CBS pathosystem during the Amazonian seasonality, it was found that the pest was detected in 10 rural properties, totaling 11 outbreaks of CBS with 439 diseased plants. Among these, 31, 38, and 370 plants were identified in 2019, 2021, and 2022, respectively, and subsequently eradicated and incinerated (Table 1) with an annual prevalence of CBS in the municipality was 5.6, 3.8, and 7.3% in 1, 2, and 8 properties, respectively. The plantations showed annual incidence rates of 1.6, 1.4, and 18.1% in the years 2019, 2021, and 2022, respectively, with a total prevalence of 5.5% in the inspected rural properties and a total incidence of 6.5% of CBS in plants.

The highest incidence (85.7%) occurred in focus 3 with only 6 diseased plants out of a total of 7 hosts on the property (Table 1) and municipality showed higher seasonal prevalence and incidence indicators for CBS during the less rainy period (81.8%) compared to the more rainy period (18.2%), where seasonal prevalence fluctuated but decreased when compared to the primary focus in 2019 (Table 1).

Due to the habit of consuming fresh citrus fruits in the Amazon region, out of the 10 focal properties, incidence was observed in only one commercial production unit (10%). Around 90% of detections occurred in domestic plantations, characterized by backyard cultivation for family consumption with random planting, no management, and no commercial purpose. In these areas, the highest incidence percentages were observed due to the proportion attributed to the low number of hosts (Table 1).

**Table 1:** Planting date, detection, incidence, and prevalence of citrus black spot during the rainy season (January-April) and dry season (May-December) in rural properties with *Citrus* species and related genera in the municipality of Eldorado do Carajás, Pará, Brazil in 2019, 2021 and 2022

| Focus | Planting Year | Detection Date | Host | Nº of Inspected Plants | Nº of Diseased Plants | Incidence (%) | Annual Prevalence (%) | Annual Incidence (%) | Seasonal Prevalence (%) | Seasonal Incidence (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2009 | 09/25/2019 | Tangerine | 50 | 31 | 62 | 5.6 | 1.6 | 12.5 | 3.6 |
| 2 | 2010 | 04/09/2021 | Orange/Tangerine | 45 | 32 | 71.1 | 3.8 | 1.4 | 3 | 1.4 |
| 3 | 1988 | 06/21/2021 | Orange/Lime | 7 | 6 | 85.7 | | | 5.6 | 1.2 |
| 4 | 1992 | 04/11/2022 | Orange/Rangpur Lime/Tangerine | 33 | 5 | 15.2 | | | 2.6 | 0.9 |
| 5 | 1995 | 08/29/2022 | Orange | 23 | 10 | 43.5 | | | | |
| 6 | 2009 | 08/30/2022 | Tangerine | 450 | 341 | 75.8 | | | | |
| 7 | 2012 | 08/30/2022 | Orange | 45 | 1 | 2.2 | | | | |
| 8 | 2010 | 08/31/2022 | Orange | 15 | 1 | 6.7 | 7.3 | 18.1 | 9.9 | 25.1 |
| 9 | 2010 | 09/01/2022 | Orange | 15 | 3 | 20 | | | | |
| 10 | 2008 | 09/01/2022 | Orange/Tangerine | 13 | 7 | 5.8 | | | | |
| 11 | 2015 | 12/14/2022 | Orange/ Rangpur Lime | 8 | 2 | 25 | | | | |
| | | | Total | 704 | 439 | | 5.5 | 6.5 | | |

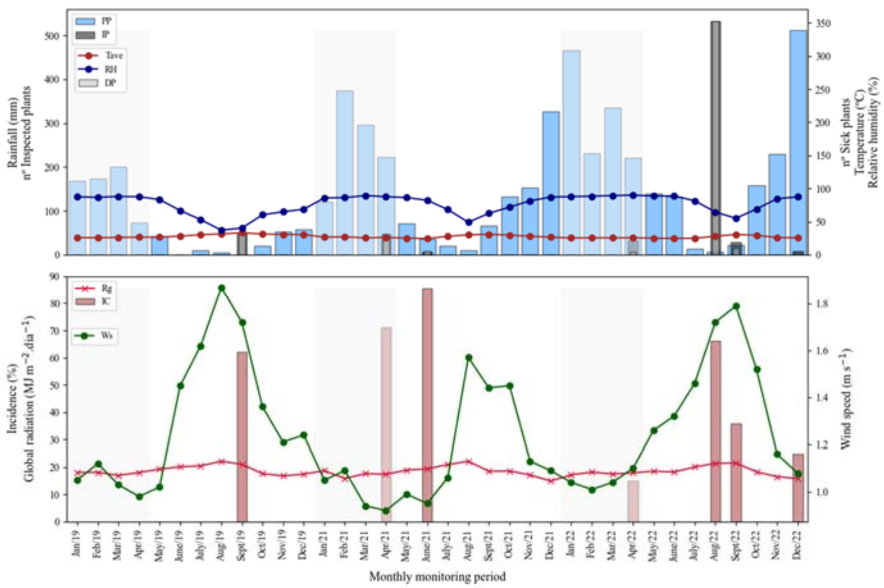


**Fig. 3:** Monthly mean values of air temperature (Tave), relative humidity (RH), wind speed (Ws), incident global solar radiation (Rg), rainfall (PP), monthly accumulation of inspected plants (IP), diseased plants (DP) and incidence (IC) on rural properties with citrus black spot in Eldorado do Carajás, Pará, Brazil, in 2019, 2021 and 2022

The environmental conditions in the municipality during the CBS monitoring surveys revealed an annual rainfall of 1,701.7 mm, along with maximum, mean, and minimum air temperatures, relative humidity, global radiation, wind speed of 40.5°C, 27.5°C, 20.9°C, 76%, 1.2 m $s^{-1}$ and 18.6 MJ $m^{-2}$ $day^{-1}$, respectively (Fig. 3).

An increase in rainfall and a temporal fluctuation in the incidence of CBS (Fig. 3) were observed. Despite the growth in the number of outbreaks in the municipality, there was a decrease in the average incidence due to the relationship between inspected plants and diseased plants, with average incidence in the outbreaks of 62, 73.1 and 61.5% in 2019, 2021, and 2022, respectively.

The prevailing wind direction in the municipality originates from the East (E) 38.7% of the time, followed by the North (N) direction with a frequency of 35.2%. During the less rainy period, there is a predominance of easterly winds (48%) with an average speed of 1.36 m $s^{-1}$, while in the rainier period, winds from the North (54.5%) prevail with an average speed of 1.04 m $s^{-1}$. Focus 1 and 6 were recorded during the less rainy period, both on the same property. A total of 372 diseased plants were identified, with maximum and average distances between affected areas of 156.9 and 41.4 m, respectively. Dispersion of the pest was observed between 2019 and 2022 (Table 1), with an increase in infections due to the lack of immediate elimination after detection by the producer. Only in 2022 did eradication occur on the property that presented the largest number of diseased plants during the analyzed period (Fig. 4).

Due to the complex and conflicting phylogeny of *Citrus* species and related genera, which complicates identification (Curk *et al.* 2016), the hosts of CBS detected in monitoring were grouped into sweet orange (52.9%),

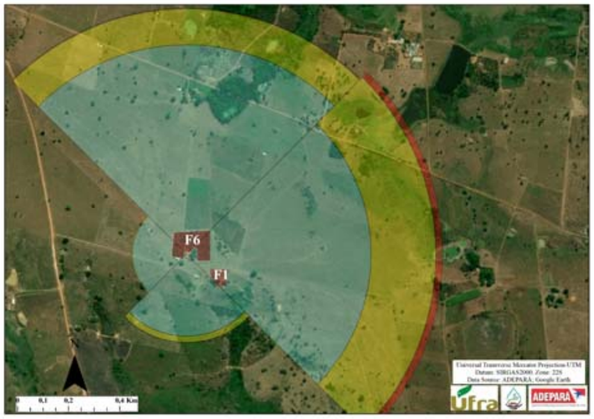


**Fig. 4:** Spatiality of wind distribution within a radius of 1000 m perifocally to the location of focus 1 (F1) in 2019 and focus 6 (F6) in 2022 of citrus black spot on rural property and the wind rose indicating the direction and wind speed of the municipality of Eldorado do Carajás, Pará, Brazil

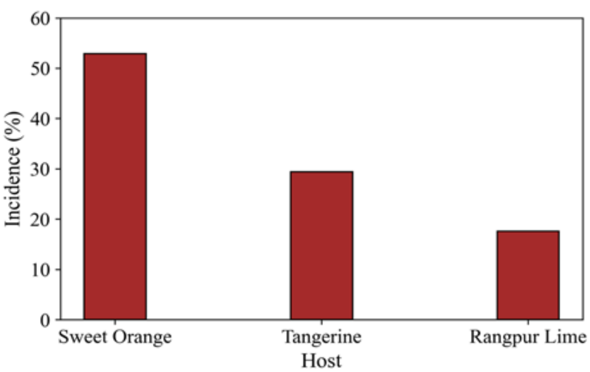


**Fig. 5:** Hosts with incidence of citrus black spot detected during monitoring surveys in 2019, 2021 and 2022 in the municipality of Eldorado do Carajás, Pará, Brazil

tangerine (29.4%), and rangpur lime (17.6%) (Fig. 5), with plantations having been established for over 10 years without irrigation systems (Table 1).

The principal component analysis, which involves the linear combination of all original variables that are independent of each other, indicated that climatic factors had a significant impact on all variables of the pathosystem, explaining 55.1% of the variation. This was followed by the monitoring factor, which influenced 22.1%. Together, these factors explained 77.2% of the total sample variance effectively. The projection of the data with axis rotation captured the structure of maximum variance and covariance (Fig. 6). The original transformed variables that showed the greatest importance and contribution to the formation of the principal components were relative humidity, inspected plants, minimum temperature, and precipitation. The dispersion of the eigenvectors revealed an interrelationship between minimum temperature, diseased and inspected plants, and the incidence of CBS (Fig. 6).

According to the MI, the incidence of CBS in rural properties at sites 1, 2 and 6 (Table 1) showed significant spatial dependence and clustered spatial patterns, with higher autocorrelation in sites 1 and 6 due to being commercial plantations with a higher density of diseased plants. However, lower aggregation was observed at site 2 in 2021, where hosts were more dispersed, with a spread of diseased plants reaching 25.5 m (Table 2). The aggregated distribution pattern of CBS, as explained by the Gaussian theoretical models (Fig. 7), indicates that the disease manifested as an extremely continuous phenomenon with a parabolic behavior near the origin, characteristic of highly regular spatial variability (Yamamoto and Landim 2013).

The disease exhibited a maximum distance of 37.4, 39.7, and 104.1 m in 2019, 2021, and 2022, respectively. The variability at a small scale was captured through sampling with a nugget effect close to zero (Fig. 7). The areas of focus 1, 2, and 6 exhibited potential disease dispersion radii of 62.3, 66.2 and 173.5 m, respectively. These radii guided field inspections and may assist in determining the variable eradication radius for controlling diseased plants.

The linear relationship between the incidence of CBS and the geographic coordinates of the focal points over the three years analyzed showed high dispersion and were not significant ($P > 0.05$) according to regression analysis. The slope parameters of the equations explained only 0.06 and 0.02% of the variation in pest incidence in the geographic area of the municipality (Fig. 8).

The high dispersion results in a behavior of random spatial distribution in plants with few diseased fruits and areas with irregular planting, such as domestic orchards, exhibiting low autocorrelation [MI of 0.4 ($P < 0.05$)] and a coefficient of determination ($R^2$ = 0.12) that precludes aggregation. The maximum distance between focal points, radius, and range of 20.5, 34.2 and 20.3 km, respectively, explains the occurrence of the disease due to uncertified propagative material in the municipality, favored by the environmental conditions of the pathosystem. In the spatiotemporal analysis of CBS at the municipal scale, a gradient of CBS incidence was observed, forming concentric areas of high incidence in the southwest and a gradient of low incidence in the northeast and southeast of the municipality (Fig. 9).

The mapping conducted using the ISD method indicated that the increase in clustered spots in the focal properties is concentrated around the BR-155 and PA-275 highways, which connect the municipality with other regions of the state of Pará and Brazil (Fig. 10), as well as along the main roads where the majority of rural properties are located, facilitating access for the sale of fruit tree seedlings through mobile vendors (Fig. 2).

The main entry routes for infected seedlings into the state of Pará are in the southeast region *via* land routes in Santana do Araguaia (BR-158), Conceição do Araguaia (BR-158/PA-287/BR-155), São Geraldo do Araguaia (BR-

**Table 2:** Analysis of the spatial distribution of plants infected by citrus black spot at the rural property level with citrus species in 2019, 2021 and 2022, analyzed by the Moran Index in the municipality of Eldorado do Carajás, Pará, Brazil

| [1]Year | [2]Area ($m^2$) | [3]NPF | [4]MI | [5]*P* value | [6]Co+C | [7]Model | [8]$R^2$ | [9]r | Radius | Spatial Pattern |
|---|---|---|---|---|---|---|---|---|---|---|
| 2019 | 1.800 | 31 | 0.87 | 0.001* | 118.87 | Gaussian | 0.90 | 34.89 | 62.29 | Aggregated |
| 2021 | 1.600 | 32 | 0.63 | 0.001* | 106.43 | Gaussian | 0.82 | 25.49 | 66.22 | Aggregated |
| 2022 | 16.200 | 341 | 0.98 | 0.001* | 13,338.06 | Gaussian | 0.83 | 102.88 | 173.51 | Aggregated |

[1]Sampling year; [2]Sampled area; [3]Number of infected plants per focus; [4]Moran Index; [5]*significant at 1%; [6]Nugget effect + spatial variance; [7]Theoretical fit of empirical semivariograms; [8]Coefficient of determination; [9]Range of infected plants (meters)

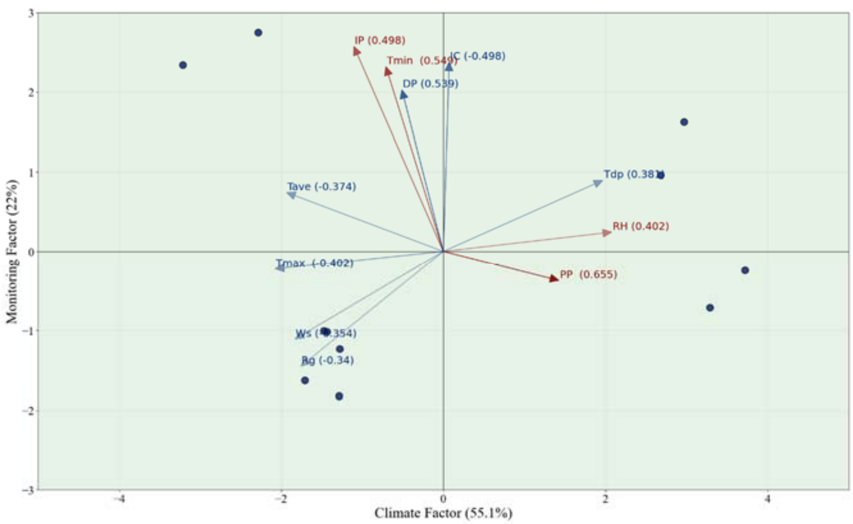


**Fig. 6:** Biplot of principal component 1 (climatic factor) *vs.* principal component 2 (monitoring factor), based on the correlation between original variables and principal components (derived from the covariance matrix) for the incidence of citrus black spot in Eldorado do Carajás, Pará, Brazil, in the years 2019, 2021, and 2022. Tmax = maximum temperature; Tave = average temperature; Tmin = minimum temperature; Tdp = dew point temperature; Ws = wind speed; Rg = incident global solar radiation; PP = precipitation; RH = relative humidity; IC = incidence; IP = inspected plants; DP = diseased plants

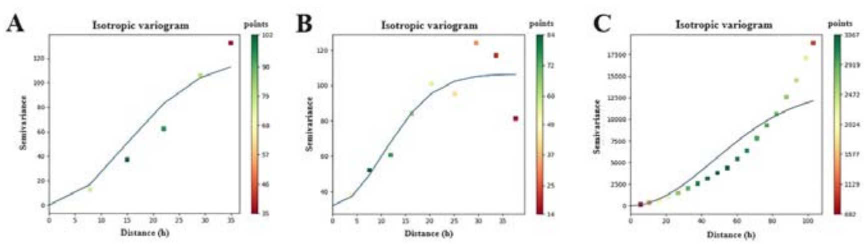


**Fig. 7:** Semivariograms demonstrating high continuity at the origin of spatial dependence for citrus black spot in properties with citrus plants in 2019 (**A**), 2021 (**B**) and 2022 (**C**). The squares represent the average of point pairs at each distance (h) for the empirical semivariograms, and the solid line represents the theoretical semivariograms obtained from fitting the empirical data using the Gaussian model

153), Palestina do Pará (BR-230), and in the southwest region *via* Novo Progresso (BR-163) and Jacareacanga (BR-230), both of which converge onto the Trans-Amazonian Highway (BR-230), a major transit route towards the citrus centers of Pará *via* BR-010 (Capitão Poço) and BR-163 (Monte Alegre) (Fig. 10).

In the northwest region of the state, entry occurs through land/water routes in Juruti *via* the Amazon River, facilitated by the intense flow of vessels and goods between the states of Amazonas and Pará with stops in Santarém, Monte Alegre, and the final destination of Belém. In the northeast region, entry takes place *via* Belém (BR-316) and Dom Eliseu (BR-010) to Irituia (PA-253), Ourém (PA-124), and Capitão Poço, with agricultural inspection posts present.

## Discussion

Although geostatistics has been used for phytosanitary analyses in citrus farming by Oliveira *et al.* (2023), this study represents the first attempt to describe the spatial pattern of CBS using the Gaussian model in the state of Pará. This finding is also corroborated by a literature review

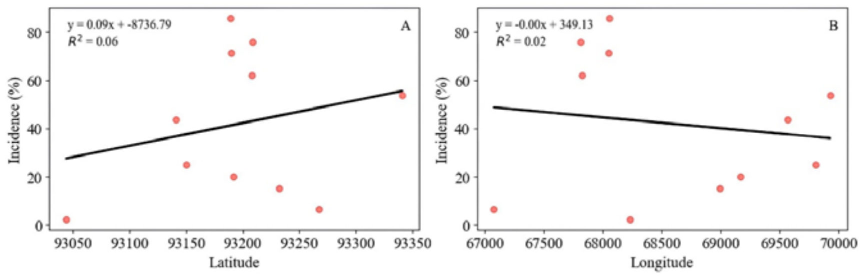


**Fig. 8:** Linear relationships between the incidence of citrus black spot regressed against latitude (**A**) and longitude (**B**) of the focal points in the municipality of Eldorado do Carajás in 2019, 2021 and 2022

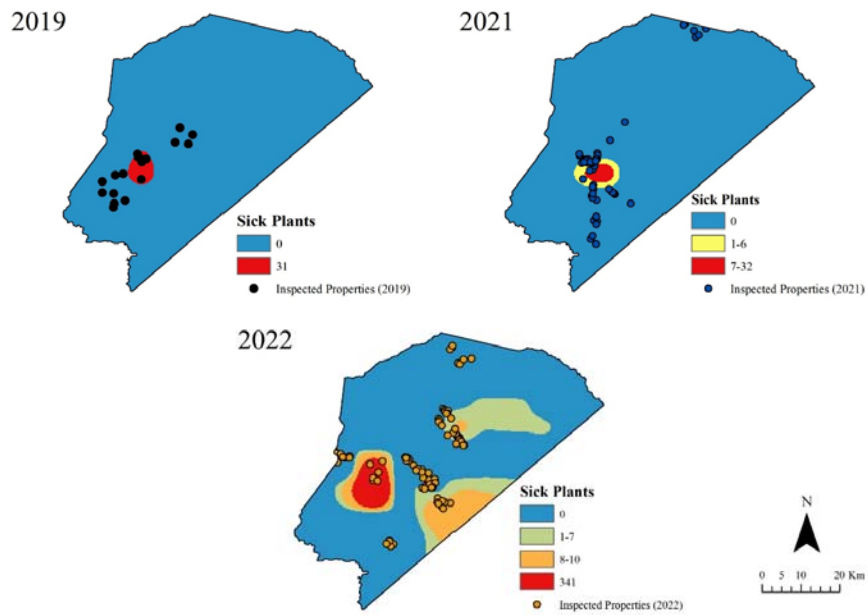


**Fig. 9:** Spatial inference of estimated incidence of diseased citrus plants with black spot in rural properties with citrus plantations in Eldorado do Carajás, Pará, Brazil, in 2019, 2021 and 2022. Regions colored blue indicate properties inspected in all years with no occurrence. Regions with clustered patches of yellow, orange and red indicate occurrence

carried out by Cardoso *et al*. (2021) on the application of geostatistics in Pará. In their research, only studies focusing on pests such as the citrus blackfly (*Aleurocanthus woglumi* Ashby), the mite (*Brevipalpus phoenicis*) and the ant (*Azteca barbifex*) were identified, all carried out in the municipality of Capitão Poço, located in the northeastern mesoregion of Pará.

The first detection of CBS in the municipality was identified on September 25, 2019, on a commercial property with two Ponkan tangerine production units, both established in 2009, in an area of 1.8 ha, with a spacing of 6 x 6 m, whose focus 1 had 50 inspected plants with an incidence of 62% of CBS. New foci were detected as monitoring surveys in the municipality progressed on properties bordering the initial focus. In 2022, the prevalence increased due to the targeted intensification of inspections on properties with focuses mapped in previous years. Ripe fruits fallen within the radius of the plant canopy facilitated the detection of the pest on the properties, considering that there is an increase in fruit drop when there is CBS in the area. It is understood that old plants with water and nutritional deficits are more affected by CBS than young plants, associated with climatic conditions due to the progressive accumulation of the fungus in the orchard (Ministério da Agricultura 2008; Silva-Júnior *et al*. 2016), which confirms the results obtained.

The rainfall in 2021 and 2022 throughout the year favored the CBS cycle, where there was rainfall in the less rainy period with higher values in the years 2021 and 2022, under the effect of *La Niña* interspersed with neutrality than in 2019 with *El Niño* (INMET 2023). The alternation observed between more and less rainy periods with wetting and drying of citrus leaves decomposing in the soil and the daily thermal amplitude are optimal conditions for the maturation of pseudothecia in the production and release of ascospores (Kotzé 1981).

The ascospores are ejected from the pseudothecia into the atmosphere, surpassing the boundary layer, and

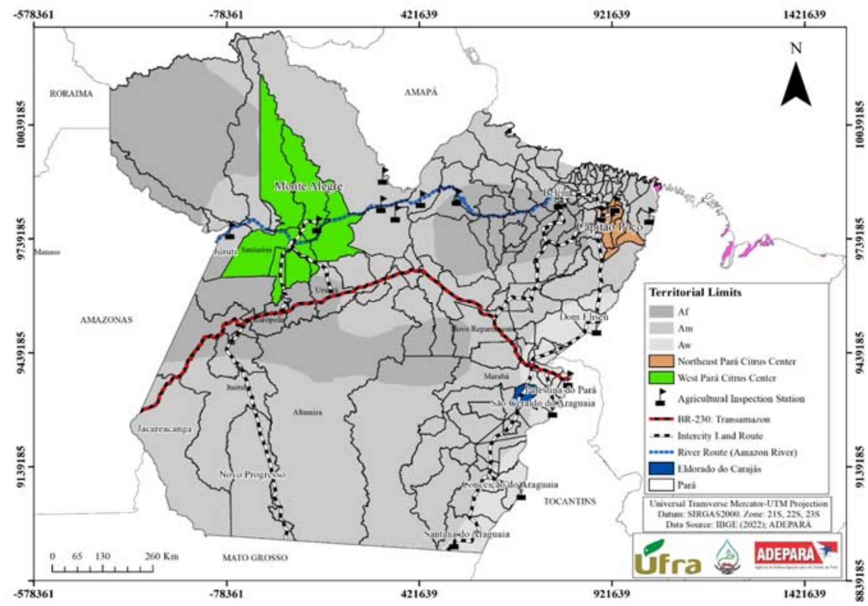


**Fig. 10:** Climate types, traffic routes, agricultural inspection post and location of the citrus hub of Northeast Pará with the highest orange production (orange color) and the citrus hub of West Pará with emphasis on the production of Tahiti lime (green color) and the municipality of Eldorado do Carajás located in the mesoregion of Southeast Pará with incidence of citrus black spot. Source: Adapted from IBGE (2022)

dispersed by the wind, where the pathogen will be deposited and cause infection. This process requires 12–24 h of free water for germination, formation of the germ tube, and appressorium, which will penetrate the plant tissue and form the mycelial mass in the subcuticular region, where the pathogen may remain quiescent depending on the fruit's age, inoculum concentration, and environmental conditions (Kotzé 1981; Feichtenberger *et al.* 2005).

Variations in severity with symptoms of hard spot to virulent due to the severe attack of the fungus by enzymes capable of degrading pectic substances of the fruit cell wall post-infection (Silva-Júnior *et al.* 2016) were observed in both the rainy and dry periods and in all collected ripe fruits. The symptom of hard spot, typically observed, appears at the onset of fruit ripening due to latent infections and can manifest up to six months after petal fall and in fruits from the first flowering (Baldassari *et al.* 2007). It presents a depressed center with dark lesions (pycnidia), light coloration at the base, more prominent edges of dark brown color that can merge and form the virulent spot within 100 to 200 days post-infection, occurring at the end of the season in high temperatures, where both symptoms serve as sources of inoculum (Silva-Júnior *et al.* 2016).

Gasparotto and Pereira (2004) detected CBS in an orchard of the Pêra Rio cultivar on the banks of the AM-010 Highway, in September 2003, in the state of Amazonas, in fruits with extremely high severity. While all citrus varieties are susceptible to CBS, especially mid-season varieties like Pêra and late-maturing ones such as Valência and Natal (Rossetto *et al.* 2011), early harvesting inhibits symptom manifestation and premature dropping of infected fruits. This is particularly crucial during warmer periods with reduced cloud cover, as it helps mitigate fruit yield reduction (Silva-Júnior *et al.* 2016).

During the surveys, no symptom expression was observed in Tahiti acid lime (*Citrus latifolia* (Yu. Tanaka) Tanaka) plants near those with CBS in the inspected properties. Tahiti acid lime is considered a commercially valuable variety resistant to CBS and is disregarded as a source of inoculum for new infections (Feichtenberger *et al.* 2005).

The minimum (20.9°C) and average (27.5°C) temperatures of the municipality, with lower values during the rainy season, favor infection. This is because ascospores are released at temperatures above 18°C (Fourie *et al.* 2013), leading to symptom expression due to the long dissemination period (40 to 180 days) and pathogen incubation (40 to 300 days) during the drier season, when there is a higher incidence of CBS. This incidence is influenced by air temperatures exceeding 20°C and longer exposure of fruits to solar radiation (Feichtenberger *et al.* 2005). For the release of ascospores and fruit infection, the presence of water is necessary (Mconie 1964). On days without rainfall, the release is lower (Lee and Huang 1973), as other elements are involved in the inoculum cycle (Hidalgo *et al.* 1997). Air relative humidity is the variable that most influences the formation of appressoria "*in vitro*," causing infection (Magarey *et al.* 2015), due to the high release of ascospores during 3 h with humidity greater than 60% (Fourie *et al.* 2013).

Correlations of the other variables with CBS incidence were not observed; however, even with decreased rainfall, air relative humidity (76%) and dew point temperature (20.2°C) may have facilitated ascospore release and fruit infection during the less rainy period (Fig. 3). These conditions minimize fungal structure desiccation at high air temperatures, highlighting the pathogen's adaptability to various post-infection climatic conditions, as seen in 2019 (Reis *et al.* 2006). Global radiation, air temperature and wind speed showed similarity and contributed to the occurrence of diseased plants and the development of plants with fruits at different maturity levels. Air temperature, leaf wetness, air humidity, and solar radiation influence the expression of CBS symptoms, where environmental conditions may be more important than the fruit ripening stage, requiring knowledge of the local environment to establish effective pest control strategies (Ninin *et al.* 2012).

According to Reis *et al.* (2006), the maturation, release, and dispersal of ascospores have a high correlation and significance with air humidity, rainfall, and low correlation with temperature, observed in areas with plantations of 'Natal' and 'Valência' oranges. Timossi *et al.* (2003) found optimal temperatures of 22.5 to 27.5ºC for the production and maturation of 90% of pseudothecia in 19 days with a 12/12 h photoperiod and a higher percentage of ascospore germination at 24ºC, which corroborates the occurrence of inoculum qualitatively and quantitatively in the climatic conditions of the inspected rural properties.

The occurrence of winds with maximum speeds averaging 5.23 m $s^{-1}$ may have contributed to the spread of the pest in the properties of focus 1 and 6. Air turbulence moves decomposing citrus leaves, which can disperse ascospores in areas both within and between orchards on the same property (Silva-Júnior *et al.* 2016). During the drier period, citrus leaves fall, while in the wetter season, there is typically decomposition of leaves on the ground, leading to increased production and dispersion of ascospores, which rely on dead citrus tissue to survive (Aguilar-Vildoso *et al.* 2002).

Sexual spores do not survive in other hosts or freely in the soil; therefore, they originate from infected plants near the orchard, infected seedlings even if asymptomatic, or through vehicles and equipment containing infected leaves (Feichtenberger *et al.* 2005; Vieira-Júnior *et al.* 2012). Regrowth or replanting areas can exhibit CBS, and even previously unaffected areas near infected zones or planted with contaminated seedlings may be affected. Consequently, the disease can spread to new areas, potentially reaching distances of 25 to 35 m and manifesting symptoms after 2 to 4 years in infected citrus plants, or even post-harvest (Silva-Júnior *et al.* 2016).

The plant inspection methodology adopted by the official phytosanitary defense service (Ministério da Agricultura 2018b) proved effective for pest detection. This underscores the importance of systematic sampling in field inspections along planting rows. Principal component analysis highlighted the significance of this variable in the pathosystem, and the near-zero nugget effect in the semivariograms indicated a small sampling scale. The spatial distribution range of CBS varied from 25.5 to 103 m, forming aggregation patches with a gradual effect based on disease spread intensity. It is recommended to conduct phytosanitary surveys at intervals shorter than this range, as it indicates the maximum distance of correlation between sample points and the spatial dependency limit of the disease in the planting area (Gama and Farias 2021).

CBS spreads to distances wherever there are susceptible hosts in the area, driven by the high potential of two dissemination mechanisms and disease density. This aggregation tendency is more pronounced in older plantings (Spósito *et al.* 2007). The presence of neighboring diseased plants increases the likelihood of infecting nearby healthy plants, with the pathogen spreading over both short and long distances, along and between planting rows, in all directions. This isotropic spread depends solely on the vector and not on direction, causing CBS to proliferate in areas cultivated with susceptible hosts (Silva-Júnior *et al.* 2016). The spread distance of CBS varies over time and can be shorter in older orchards due to the larger size of the plants, which reduces wind circulation, as well as the arrangement of the plants in the field (Spósito *et al.* 2007). The distances between pairs were 7.2, 4.3, and 5.5 m in 2019, 2021, and 2022, respectively. In Pará, common planting arrangements for citrus species include 4 × 5, 5 × 5, 6 × 6, 6 × 7 and 7 × 7 m, influencing the microclimate. These arrangements did not prevent CBS infections from entering the inspected areas.

Laboratory diagnostics confirmed the presence of conidia of the fungus *P. citricarpa* and the absence of ascospores in the fruit samples. The asexual form identified is responsible for auto-infections in fruits, leaves, and branches, increasing disease severity. This is facilitated by rainwater or non-localized irrigation systems, which support the aggregated pattern observed (Kotzé 1981; Spósito *et al.* 2007).

Asexual spores are produced in pycnidia and only on fresh fruits, which are enveloped in a clear, water-soluble mucilage (cirrus) that protects them from desiccation. These spores are dispersed downward on the plant and, upon contact with water, dissolve to release a conidial suspension. This suspension flows down the fruit, spreading 54 to 77 cm from the infected fruit (Spósito *et al.* 2011; Silva-Júnior *et al.* 2016). Pycnidiospores cause infection through the stomata of green fruits, producing false melanosis symptoms due to phenol accumulation in the guard cells and surrounding sub-stomatal chamber cells. This prevents the formation of subcuticular mycelial masses, instead developing in the epicarp and mesocarp, causing cell lysis, hyperplasia, and mesocarp disarray (Baldassari *et al.* 2007) at temperatures between 4 and 37ºC and high humidity (Er *et al.* 2013). In the municipality, maximum temperatures and humidity were observed at 40.5ºC and 89.9%, respectively.

Spatial inference using the deterministic ISD method, which spatially represents areas with low point density based on purely geometric criteria where distances are Euclidean and do not provide uncertainty measures (Silva *et al.* 2019), proved to be more continuous and better identified focus areas and pest population intensity each year analyzed compared to ordinary kriging, which showed lower statistical performance. Spatiotemporal mapping assisted in identifying and controlling the pest by showing its progression in the field through clustered patches (Gama and Farias 2021). This method could also support the delineation and publication of supplementary legislation for affected areas (Ministério da Agricultura 2008).

The two disease dispersion mechanisms pose a threat to neighboring properties. In the state of Pará, properties with outbreaks of the pest are placed under quarantine measures, which include banning the planting of new *Citrus* genus seedlings and prohibiting the removal of citrus fruits and any vegetative propagation material from the affected property. Additionally, adjacent properties at risk of disease occurrence are also subject to restrictions (Ministério da Agricultura 2008, 2018b; Agência de Defesa Agropecuária do Pará 2010).

In the state of Pará, the official phytosanitary measure for controlling the establishment and spread of CBS and minimizing the socioeconomic impact on the citrus production chain is the eradication of infected plants (Agência de Defesa Agropecuária do Pará 2010). In the literature, fruit collection and the removal and burning of all leaves every 30 days before new fruit formation are reported as measures to control and reduce CBS incidence, yielding significant results in an 8-year-old Valencia orange orchard without chemical control (Silva-Júnior *et al.* 2016). Furthermore, the use of windbreaks with non-host plants and ecological mowing as a physical barrier have also been employed to prevent ascospore dispersion and reduce fruit infections (Feichtenberger *et al.* 2005).

According to the Koppen classification, the state of Pará has the climate types Af, Am and Aw with altitudes of 61, 107 and 180 m, average annual temperatures of 27.7, 27.5 and 27.3ºC, with maximums of 29.4, 29.4, 29.2ºC, minimums of 24.1, 24, 25.2ºC and average annual radiation of 227, 215 and 161 W $m^{-2}$, respectively (Alvares *et al.* 2013). The range of climatic conditions in Pará is favorable for ascospores (15 and 35ºC) and pycnidiospores (10 and 35ºC) which may allow the occurrence of CBS infection (Magarey *et al.* 2015).

The state of Pará encompasses a vast territorial area of 1,245,870.704 km² (IBGE 2022), posing challenges in containing clandestine activities. Thus, phytosanitary education plays a crucial role in guiding rural producers and other stakeholders in the production chain regarding the purchase of fruit seedlings with mandatory phytosanitary documentation, which verifies the traceability and health of the genetic origin of the propagative plant material (Agência de Defesa Agropecuária do Pará 2023). This education is essential to deter the illicit sale of seedlings that do not comply with established standards, characterized by off-site trading in Pará municipalities. Additionally, it shares responsibilities with society regarding the risks and impacts of pest dissemination in the Pará territory (Agência de Defesa Agropecuária do Pará 2010).

Due to the spread of CBS being associated with the transport of infected leaves and propagation materials such as citrus seedlings and rootstocks to areas with susceptible hosts and favorable environmental conditions, fresh fruits with conidia presence are marketed within the Brazilian territory, including to disease-free Federal Units. However, these fruits must be free from leaves and branches and originate from production units registered in the CBS risk mitigation system inspected by official control (Ministério da Agricultura 2008).

In order to prevent the introduction of the pest into citrus-growing areas through detected shipments that are rejected, the export of symptomatic citrus fruits to countries like those in the European Union is prohibited. For exports, the exporting country is required to provide a phytosanitary certification declaring the pest's absence from cultivation, storage (*packinghouse*), transportation, and handling by registered operators involved in fruit handling, as part of an official control program (Silva-Júnior *et al.* 2016). In the state of Pará, certification and accreditation of professionals for official pest control are carried out by ADEPARÁ (Agência de Defesa Agropecuária do Pará 2010).

The states of Amazonas, Bahia, Espírito Santo, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pernambuco, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo have reported occurrences of the pest (Ministério da Agricultura 2018a). Considering the risk of disease introduction into monocultures in the state of Pará from these Federal Units, identifying the main routes of pest introduction is crucial strategic information for phytosanitary management. Entry of *Citrus* genus propagative material into citrus production areas is prohibited, and authorization from ADEPARÁ is required for the entry of such materials from states without the presence of the pest (Agência de Defesa Agropecuária do Pará 2023).

The best control measure for CBS is prevention to safeguard the plant health of citrus production areas in northeastern Pará (Irituia, Capitão Poço, Ourém, Garrafão do Norte, and Nova Esperança do Piriá), located 667 km away from the outbreaks, and the citrus production hub in western Pará (Monte Alegre, Alenquer, Santarém, Mojuí dos Campos, Belterra, Prainha), situated 1.124 km away (Agência de Defesa Agropecuária do Pará 2017).

The spread of the pest in orchards in Pará, especially in old plantations, may require 4 to 5 applications of copper fungicides at intervals of 21 to 28 days at a dose of 50 to 90 g of Cu 100 $L^{-1}$, which causes severe symptoms of phytotoxicity in the bark (Feichtenberger *et al.* 2009) and depreciates fresh fruits, the main form of marketing of the product in Pará (FAO 2022). In addition, the application of strobilurins at intervals of 30 to 45 days at a dose of 3.8 g of a.i./100 L or Bordeaux mixture, starting with the fall of 2/3 of the petals, observing the onset of rains, varieties and fruit development (Silva-Júnior *et al.* 2016).

Surveillance, coupled with eradication of diseased plants through spatiotemporal monitoring in orchards *via* phytosanitary surveys, nursery inspections, and regulation of plant material transit, plays a crucial role in stimulating public policies for fruit seedling production in certified and protected nurseries. Additionally, it aids in the planning of official phytosanitary management to legally prevent the introduction, establishment, and spread of plant pests, thereby minimizing vulnerabilities posed by the CBS threat to Amazonian citrus farming, especially subsistence agriculture. The aim is to protect the health and sustainable development of Pará's citrus production chain.

## Conclusion

Citrus black spot showed an aggregated pattern with spatial dependence modeled by the Gaussian model in domestic and commercial plantations at the property scale, and at the municipal level, low prevalence in properties, with a random spatial pattern in old plantations with a low number of hosts. Seasonality caused infections, caused by conidia, in susceptible hosts with higher prevalence and incidence in the less rainy period. The inverse square of the distance inferred the spatiotemporal occurrence of the disease, providing guidelines to identify geographic areas with pest occurrence and fruit seedling marketing routes, assisting strategic control actions and minimizing vulnerabilities to the introduction of fungal diseases in citrus producing areas of Pará.

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## Author Contributions

VBM participated in all stages of research, writing and final review of the article, RJMJ, LPP, NVDS, LMMDM and PJDOPDS planned the experiments, RJMJ, ECOV, ARP and RADSC collected information in the field, RJMJ, LPP, DLPC, GSTF and PJDOPDS interpreted the results, MATL and PJDOPDS wrote and discussed the data and DLPC, JTSDS, LASC, PJDOPDS statistically analyzed the data and made illustrations.

## Conflict of Interest

All authors declare that there is no conflict of interest

## Data Availability

The data presented in this study will be available upon fair request to the corresponding author

## Ethics Approval

Not applicable to this paper

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