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Space-time analysis, severity of the wilt disease in escabeche pepper (*Capsicum baccatum* var. Pendulum) and identification of the causal agent (*Phytophthora capsici* L.) under subtropical climate conditions in Peru

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Abstract

Phytophthora capsici is an aggressive pathogen in escabeche pepper on the Peruvian coast. Root rot has a strong correlation with humidity and environment. Disease behavior was evaluated epidemiologically using spatiotemporal variables. Severity was evaluated according to the advance of the secondary symptom according to grades 1 to 5. Then, coordinates of each plant were established by photogrammetric survey of a field with 1705 escabeche pepper plants. For temporal analysis, severity was adjusted to an exponential model ($R^2 = 0.909$) and incidence to a Gompertz model ($R^2 = 0.921$) that detected an initial delay of the disease due to temperature. For the spatial analysis, the Global Moran Index (II) showed a high spatial dependence of the disease reaching a peak of 0.4 and 0.7 for severity and incidence, respectively. Also, heat maps related to the Local I were generated from which an initial source of infestation was determined where the furrow irrigation started in random infestations. Then, the infestation spots were settled in areas of surface water accumulation. Also, rhizosphere samples were collected per plant by degree of severity on V8 or CMA whit PARB and PDA-A selective medium. As a result, significant differences were obtained between grade 1, grade 2, 3, 4 and grade 5. In addition, the effect on yield was significant for plants with grade 4 and 5 with respect to fruit weight (22.3 and 18.5g/fruit) and weight per plant (509.5 and 371.8g/plant), respectively.

Keywords: Epidemiología; Espaciotemporal; Phytophthora capsici; Capsicum.

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1. Introduction

Capsicum baccatum L. is one of the chilli pepper species cultivated mainly in Latin America. This species has a high variability of traits closely related to the fruit, disease resistance and bioactive compounds. The origin of *C. baccatum* is not yet fully defined and accepted, however, studies indicate that this crop has its origins in Bolivia and southern Peru. *C. baccatum* can be classified into three botanical varieties: *C. baccatum var. pendulum* (Willd.), *C. baccatum var. baccatum* silvestre (formerly *C. microcarpum*) and *C. baccatum var. umbilicatum*. (Gomes et al., 2021).

Phytophthora capsici is a pathogen that was isolated from chilli pepper plants in New Mexico in 1918 and formally described in 1922. This pathogen belongs to the group of soil-borne Oomycetes (pseudofungi) affecting *Capsicum*, *Solanaceae*, *Cucurbitaceae* and *Malvaceae* crops worldwide. *P*. *capsici* infects different parts of plant tissue (stem, roots, leaves and fruits), causing significant damage to crops, and may even damage the entire field (Kaur et al., 2024; Xu et al., 2024).

In this sense, the importance of evaluating the development of the disease caused by *P. capsici* in the field has to be demonstrated in conjunction with the concentration of inoculum present and its infective capacity (**Serrano-Pérez et al., 2017**); therefore, the present study consisted of the geostatistical monitoring and evaluation of the severity of the root rot disease in escabeche pepper caused by *P. capsici* in a traditional cultivation space. At the same time, it is taken into consideration that there are deficiencies in the understanding of the infection process and pathogenic mechanism of *P. capsici* (Li et al., 2020).

The spatiotemporal analysis of diseases can estimate the threats that exist in different pathologies. Also, understanding the spatial distribution of the pathogen to be studied helps to estimate the interrelationships of inoculum concentration and disease severity (**Balanagouda et al., 2021**). There is a problem around the epidemiological way of propagation of plant diseases both in their relationship from individual to individual, amount of water, susceptibility of the host and characteristics of the system. Therefore, it is necessary to estimate this irregular disease progress by the best geostatistical tools (**Belan et al., 2018**).

The development of the following objectives directed the pathway of this research: To analyze the spatiotemporal and epidemiological behavior of Phytophthora capsici in an escabeche pepper field under subtropical conditions. Specific objectives were: To evaluate the incidence and severity of the root rot caused by *P. capsici* in the escabeche pepper crop by adjusting the disease curve; to evaluate the temporal and spatial behavior of the escabeche pepper root rot spots in the field in the experimental area through geostatistical analysis; to correlate the concentration of the P. capsici inoculum in the soil with the areas where the escabeche pepper crop was affected with the disease in the previous crop season and to determine the influence of the severity of P. capsici on the yield of escabeche pepper under subtropical conditions.

2. Methodology

2.1 Study area

The present research was carried out in the experimental fields of the Universidad Nacional Agraria La Molina (UNALM) in which it was considered that the causal agent of the wilt disease in Capsicum exists in the soil, since it was observed in previous crops

Table 1

Soil analysis of the experimental area

and as **González et al. (2013)** did in a similar study. Prior to sowing the crop, tillage work was carried out such as harrowing, ploughing and furrowing, using furrow irrigation with a frequency of 10 to 14 days at 1m spacing between furrow and 0.5 m between plant, for a total of 1800 plants, with an area of 876 m². An intensive mapping of the location of each plant was carried out, generating a map with 1705 plants previously marked in the field using a DJI Phantom 4 v2.0 drone, this data obtained using ArcMap v10.8. Soil type is loamy with a medium electric conductivity and high levels of calcium. The soil had good characteristics for escabeche pepper crop (**Table 1**).

2.2 Evaluation of incidence and severity of the disease

Disease assessment was measured each week in the form of incidence and severity. The incidence of the disease was evaluated on a scale from 0 to 1; being 0 when the plant was healthy; and 1 when the plant presented the disease; using the data, the proportion of diseased plants over the total number of plants was found (Bellini et al., 2020; Saltos et al., 2021). For severity, it was evaluated on a modified scale from 1 to 5 for all plants according to Ozyilmaz (2020) (Table 2).

Table 2

Degrees of severity to evaluate the secondary symptom of root rot caused by *P. capsica*

Grade	Description
1	No visible disease
2	Wilting of the most mature leaves (lower third)
3	Wilting of all leaves except the upper part of the plant
4	Wilting of all leaves
5	Defoliation and / or death of the plant
Source: Base	d on Ozvilmaz (2020).

Variable Value Methodology pH (1:1) 7.8 Measurement on the potentiometer 1:1 Measurement of the CE 1:1, soil:water CE (1:1) (dS m⁻¹) 1.93 4 80 CaCO3 (%) Dietrich-Fruhling calcimeter M.O. (%) 65 Walkey y Black P (ppm) 10.40 Olsen modified K (ppm) 274 Ammonium acetate extraction Arena (%) 52 Hydrometer Limo (%) 24 Hydrometer Arcilla (%) 24 Hydrometer Textural type Fr Hvdrometer CIC 13.12 Saturation with ammonium acetate Ca+2 942 Replacement with ammonium acetate Mg⁺² 2.93 Replacement with ammonium acetate K+ 0.56 Replacement with ammonium acetate Na⁺ 0.21 Replacement with ammonium acetate A|+3 + H+ Yuan 0 Sum of cations 13.12 Suma of bases 13.12 Saturation of bases (%) 100

Note: Physical and chemical properties of the soil of the experimental plots. Source: Soil analysis lab – UNALM.

2.3 Temporal analysis

Average temperature data measured by the Von Humboldt Meteorological Station (OVH), located in La Molina-Lima Peru, were taken from the web page of the National Service of Meteorology and Hydrology (Senamhi, 2023), in relation to the phenological stages of the crop (Figure 1). Incidence and severity data were adjusted to exponential, logistic, monomolecular and Gompertz models. These adjustments were evaluated by using statistical parameters such as the coefficient of determination (R2), cubic correlation coefficient (CCC) and the residual standard error. For this, the "epifitter", "readxl" and "tidyverse" packages were used in R software (Alves & Del Ponte, 2021; González-Concha et al., 2021).

2.4 Spatial Analysis

Degree of severity through the time was recorded for each plant. Heat maps that delimit disease areas were made using the Surfer v.16 program using the severity kriging method interpolated from the orthophoto of the field (**Gasparoto et al., 2018**). Thus, the analysis was based on estimating the aggregation of the disease through the Global and Local Morán Index that can be used in categorical variables.

Analysis of the results were based on the closeness of the values from -1 to 1 where values close to -1 indicate uniformity, values equal to 0 indicate randomness and values close to 1 indicate aggregation. These analyzes were carried out in R software using the "readxl", "writexl", "sp" and "spdep" libraries (González-Concha et al., 2021). Likewise, the Local Moran Index can display groups of high values (High-High or HH), groups of low values (Low-Low or LL), a high value surrounded by low values (HL), or a low value surrounded by high values (LH) (Kozonogova & Dubrovskaya, 2020; Lord et al., 2021).

2.5 Influence of *P. capsici* inoculum concentration on the disease

Random sampling was carried out with respect to the degrees of severity using the scale according to Ozyilmas (2020). Rhizosphere samples of 100g were collected according to optimal fixation per infected plant and from the samples obtained, 1g of soil was extracted at 5 replicates per sample (Sosa-Herrera et al., 2019). Then, using a selective medium for P. capsici made with plant extract V8 or CMA plus PARB (Pimaricin, Ampicillin, Rifampicin, Benomyl), 1 g of soil was placed and incubated at 25 °C for 3 days. Then, after observing the growth of colonies characteristic of Oomycetes, we proceeded to repot these colonies on PDA-A (Potato Dextrose Agar - Ampicillin) universal medium for fungi to morphologically identify the presence of P. capsici from other soil microorganisms. The experimental design was a DCA of each severity scale with 12 replications. Results were analyzed in R software using the "nortest", "car", "Imtest" and "readxl" libraries (Bi et al., 2012; Villarino et al., 2021).



Figure 1. Temporal behavior of mean temperature under subtropical conditions in relation to crop phenology in coastal Peru. *DAT (Date After Transplanting).

2.6 Disease effect on yield

Escabeche pepper was harvested from December 24, 2022, to February 7, 2023, every week. Fruits were almost orange to totally orange. Fruit fresh weight, number of fruits and weight per fruit were taken pr plant. Finally, ANVA and Tukey test were performed for multiple mean comparisons. All data analyzes were carried out in R software using the "nortest", "stats", "car", "Imtest" and "readxl" libraries (**Rai & Guest, 2020**).

3. Results and discussion

The BLAST result indicates 100% identity with the species *Phytophthora capsici* Leonian.

3.1 Temporal analysis of chili root rot caused by *P. capsici*

3.1.1 Meteorological conditions

In Figure 1 can be observed the increase in average temperatures for October 29 (184 DAT) with respect to favorable conditions for the pathogen. It is estimated that the temperature variation before 184DDT was between 14 to 16 °C with a qualitative peak between 16 to 18 °C for 184 to 226 DDT, corresponding to the ripening of the fruits. Finally, from 226 to 276 DDT corresponding to the harvest, temperatures were from 18 to 22 °C with a tendency to increase.

3.1.2 Severity

The curve of the progress of the disease (Figure 2) with respect to severity shows a significant increase starting December 22 (238 DAT), which shows exponential growth. At this moment, it coincides with the rise in temperatures shown in Figure 2 with average temperatures of 18 to 20 °C compared to the period from July 29 (92 DAT) to October 29 (184 DAT) with average temperatures of 14 to 16 °C. Likewise, curve fitting was performed using the "epifitter" package in R, the result of which had a greater correlation with an exponential model according to Table 3. The severity scale was assessed according to Ozyilmaz (2020) as shown in Figure 3.







Figure 3. Severity scale. Grade 1, healthy plant; Grade 2, wilting of old leaves; Grade 3, wilting ¾; Grade 4, total wilting; Grade 5, defoliation and death.

Table 3

Results of the adjustment for the Exponential, Logistic, Gompertz and Monomolecular model for the severity curve, severity curve, under subtropicals conditions

	CCC	R ²	RSE	
Exponential	0.9526	0.9094	0.0943	
Logistic	0.9430	0.8921	0.1233	
Gompertz	0.9228	0.8567	0.0657	
Monomolecular	0.8751	0.7779	0.0298	

Note: Where CCC is Cubic Correlation Coefficient, R^2 is the correlation coefficient and RSE is the Residual Standard Error.

The exponential model assumes a disease increase rate (dy/dt) proportional to the disease index (y) from which it explains the early stages of polycyclic diseases (**Singh et al., 2019**). Likewise, the model indicates that the disease has a constant increase with respect to time. The exponential model explains that it can be estimated how the disease intensifies over time (**Naseri, 2022**). The interpretation of this model indicates a slow initial growth followed by an unlimited increase (**Naseri & Nazer Kakhki, 2022**).

3.1.3. Incidence

The curve of the disease with respect to incidence shows an intermediate growth of the disease for day 120 to 238 DAT. Therefore, there is a major phase of exponential growth of the disease from approximately 238 to 273DDT, according to **Figure 4**. However, there are inflection points in the incidence of the disease with a behavior similar to the severity curve. However, the incidence is described in a Gompertz pattern according to **Table 4** which is typical for polycyclic diseases. Evaluating the best fit model allows us to compare epidemics to develop and set better strategies (López-Vásquez & **Castaño-Zapata, 2022**).



Figure 4. Curve of the progress of escabeche pepper root rot disease with respect to incidence.

Table 4

Fitting result for the Gompertz, Logistic, Mono molecular and exponential model for the incidence curve of root rot of yellow chilli pepper

	CCC	R ²	RSE
Gompertz	0.9591	0.9214	0.2518
Logistic	0.8905	0.8026	1.1310
Mono molecular	0.8669	0.7651	0.1750
Exponential	0.8647	0.7616	0.9752

Note: Where CCC is Cubic Correlation Coefficient, R^2 is the correlation coefficient and RSE is the Residual Standard Error.

A general trend is established for the incidence and severity of a delay of the disease in the initial development from the beginning of the season until 120 DAT according to Figures 2 and 3. This can be explained by the environmental factor since, despite the high humidity seen in areas of water accumulation due to furrow irrigation, the disease did not thrive because temperatures did not exceed the threshold of 16 °C. Since low surrounding radiation affects air temperature, soil temperatures in the root area are similar. In this way disease development remained stable during that period (Kabir et al., 2022). On the other hand, the decrease in these values over time coincides with the absence of irrigation. This agrees with similar studies on the spatio-temporal behaviour of species of the genus Phytophthora such as P. nicotianae, P. insfestans and *P. capsici*, coinciding with the fact that disease outbreaks are more frequent in areas with greater water accumulation (Rojas et al., 2019; Fernandez et al., 1999; González et al., 2013). On the contrary, there are decreases in severity and a similar behavior to incidence. This effect should be shown inversely because of the lack of water, which subjects the plants to water stress, so it could be explained in relation to the inoculum load that the plant receives due to irrigation and the resistance of the plant when the accumulation of water disappears. Also, growth rates by epidemic phase were established (Table 5), meaning how much is the progression of the disease over time according to the slope of the curve in each period (González-Concha et al., 2021).

Table 5

Disease growth rates with respect to the epidemic phases of root rot of escabeche pepper

Epidemic	Growth	n rates	- Development time
stage	Severity	Incidence	-Development ume
Initial	0.0017	0.0105	0-120DDT
Growth	0.0024	0.0077	120-238DDT
Exponential	0.0187	0.0282	238-273DDT

3.2 Spatial analysis of chili pepper root rot caused by *P. capsici*

The movement of the hottest and coldest spots is explained according to the stages of the disease given in the temporal analysis. It is, therefore, observed that within the population dynamics of the escabeche pepper, the disease affected under conditions of heterogeneity of factors in a lesser degree for secondary infestations than for the most important clusters (Vahamidis et al., 2020). Furthermore, according to Figure 5b, the initial focus was located where the furrow irrigation started being the main starting point of the disease. For the experimental field, it was reported that there was irrigation control against the presence of *Phytophthora capsici* in the 2020 tomato season (**Casimiro, 2022**). In addition, for the escabeche pepper season during 2021, the presence of *P. capsici* was evaluated in the field (**Huacamayta, 2021**). The random way of spread of the pathogen according to **Figure 5a** may be due to survival over time. Thus, the presence of weeds is an alternative host for *P. capsici* that, although it has low colonization, can generate an exponential infestation. Likewise, **Figure 6** shows a general relationship of disease progression with the direction of irrigation water (**Dye & Bostock, 2021**; **French-Monar et al., 2006**).

It was observed that the Global Morán Index grew as the disease established itself with two important peaks, at 200 DAT and the one that corresponds to the peak of the disease at 240 DAT (**Figure 6**). The presence of positive data from the Moran index explains that there is an increasing aggregation of the disease. This implies that, naturally, the spatial distribution of soil pathogens tends to be in spots (Ulacio-Osorio et al., 2012).

The use of the local Moran Index allows the analysis of these parameters using parameters of hot zones and cold zones (high-high) and (low-low) from which greater importance is given to significant p values (p < 0.05) (Huded et al., 2022). For the initial phase of the disease, a first hot zone can be denoted where the furrow irrigation starts, in the northern part of the field. However, the low-high values surrounding random areas where the furrow irrigation initiates in Figure 7a and 7b are denoted as atypical due to a low global Morán Index. In short, the beginning of the epidemic occurred in a first focus in the hot zone at the beginning of irrigation (Vahamidis et al., 2020).



Figure 5. Heat maps regarding the decline severity for the first disease phase being (a) May 27 (29DAT) and (d) August 26 (120DAT). For the second phase (c) September 23 (148DDT) and (d) November 24 (210DDT). For the third phase: (e) January 19 (266DDT) and (f) January 26 (273DDT).



Figure 6. Degree of disease aggregation of the Global Morán Index of periodic evaluations of escabeche pepper root rot disease.

The development of the growth phase tends to a high initial growth followed by a lack of growth of the disease. Therefore, this is due to the temperatures that affect the development of the disease. However, there is a peak shown in **Figure 7d** that is explained by the drought period because during that season the general cleaning of the irrigation canal was carried out and therefore the provision of water to the crop fields was suspended. This predisposes plants to water stress and thus accentuates the symptoms caused by the pathogen (**Del Castillo Múnera et al., 2019**). **Figures 6e** and **6f** show that in the exponential phase of the disease grouping of hot zones (high-high) and cold zones (low-low) of

established severity occurs (**Balanagouda et al.**, **2021**). Furthermore, the observation of atypical high-low or low-high data indicates a disconnection between blocks or spots that act as protection belts. Thus, each spot or block have their own characteristics favorable to the development of the disease (**Di lorio et al., 2019**).

3.3. Molecular analysis of the causal agent (P. capsici) isolated from the root of the chili plant

Molecular analysis was performed at the "Umbrella Genomics Company" for the ITS region using primers ITS1F and ITS4, obtaining the following consensus sequence:



Figure 7. Distribution map of significant values of the local Morán Index for the first phase of the disease being (a) May 27 (29DAT) and (b) August 26 (120DAT). For the second phase (c) September 23 (148DDT) and (d) November 24 (210DDT). For the third phase: (e) January 19 (266DDT) and (f) January 26 (273DDT), under subtropicals conditions. In red are the High-High (HH) points, in pink are the High-Low (HL) points, in light blue are the Low-High (LH) points and in blue are the Low-Low (LL) points.

3.4. Influence of *P. capsici* inoculum concentration on the disease

Results for the inoculums concentration indicate the positive values for *Phytophthora capsici* by severity degree. Thus, the assumptions were made where it was found that for the performance of the ANVA there were significant differences between grades 1 and 5 that, in the performance of the Tukey test, the necessary significance was reached to conclude the existence of differences between grades according to the **Table 6**. Therefore, the dependence of disease development is subject to the inoculum concentration.

The evaluation regarding inoculum concentration is usually evaluated by regression analysis. There is, then, an analysis of how much of the severity increases with respect to the inoculum concentration (Silva et al., 2020). However, under the premise of obtaining more precision and objectivity, parametric test analysis can be used. This is what the present study seeks: testing subjectivity by estimating the differences between inoculum and severity (Del Ponte et al., 2017).

Table 6

Average number of positive cases of *P. capsici* by degree of severity

Severity	Average number of <i>P. capsici</i> positive cases per plant		
Grade 1	2 a		
Grade 2	3,5 ab		
Grade 3	3,8 ab		
Grade 4	5 ab		
Grade 5	4.8 b		

Note: Equal letters indicate no significant difference at 5% significance level.

Table 6 shows that the differentiation is denoted in grade 1 and in grade 5, from which it can be deduced that there may be a healthy grade, an intermediate grade, and a severe grade of the disease instead of five. Likewise, a percentage ranking can be proposed for this degree of severity considering what is described in **Table 2** to avoid type II errors (**Bock et al., 2021**).

3.5. Disease effect on yield performance

The total production of escabeche pepper for the experimental field was 678.27 kg evaluated in an area of 876m2. In this way, a total yield of 7.62 Tn/Ha resulted. This relationship must be considered due to the impact on the physiology of the plant that is seen in the performance. Thus, the objective of the epidemiological analysis is conditioned by the response of the loss of yield (**Pangga et al., 2023**). Likewise, since severity measures the degree of progression of the disease, it is an important factor to predict performance (**Esgario et al., 2020**).

For the number of fruits parameter, according to the Kruskal-Wallis test, no significant differences were found by grade according to **Table 7**. Hence, plants of the genus Capsicum are not sensitive to this variable in performance (**Abbasi et al., 2020**). On the other hand, the Tukey Test carried out for the weight of fruits per plant and for the average fruit weight differences were found between grades 4 and 5 with respect to the others according to **Table 7**. In this way, knowing that the yield is related to growth, *P. capsici* affects height, stem width, leaf width and leaf length because it reduces the development capacity of the plant (**Retes-Manjarrez et al., 2020**).

Table 7

Relation between root rot severity and average fruit number per plant, fruit weight per plant and average fruit weight in escabeche pepper

Severity	Average number of fruits per plant	Fruit weight per plant (grams)	Average fruit weight (grams)
Grade 1	22.215 a	555.727 a	26.131 a
Grade 2	21.361 a	532.982 a	25.052 ab
Grade 3	21.4483 a	511.476 a	25.413 ab
Grade 4	23.494 a	509.494 ab	22.299 bc
Grade 5	21.015 a	371.759 b	18.416 c

Note: Equal letters indicate no significant difference at 5% significance level.

4. Conclusions

The spatiotemporal and epidemiological behavior of *Phytophthora capsici* in an escabeche pepper field under subtropical conditions was disseminated from one spot to several in a grouped manner in correlation with temperature and furrow irrigation. The curve of the disease in relation to incidence and severity were adjusted to Exponential and Gompertz models, respectively, finding three phases of the disease: Initial phase, Growth phase and Exponential phase. The influence of the severity of the escabeche pepper root rot disease under subtropical conditions on the yield was significant for degree 4 and 5 of the disease progression.

This study shows that *P. capici* on escabeche pepper has a radical behavior in the presence of high temperatures and water accumulation. This suggests that proper water management, avoiding flooding, can reduce the incidence of the disease. However, if this is not achieved, it would be possible to eliminate the escabeche pepper plants close to the water accumulation points, to perhaps prevent these plants from spreading the disease to their neighbor plants, thus reducing yield losses due to the advance of the disease.

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