

# Symptomatological Characterization and Epidemiological Considerations of Persian Lime Death Syndrome in Commercial Orchards

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## Abstract

The Persian lime death syndrome (PLDS), also known as wood pocket, has reemerged as a serious problem affecting Persian lime production in the Martínez de la Torre region of Veracruz, causing tree mortality in commercial orchards. This study aimed to characterize the symptom progression and epidemiology of the syndrome in commercial orchards in the region, with the goal of developing a systematic description to better understand its etiology and facilitate its diagnosis. The incidence of this abiotic disease was assessed through visual inspection, recording the typical foliar chlorosis in all trees over a four-month period. Epidemic dynamics were analyzed by fitting the data to the flexible Weibull model ( $y = 1 - [t/b]^c$ ), estimating initial incidence ( $y_0$ ), final incidence ( $y_f$ ), disease progress rate ( $b^{-1}$ ), and the area under the disease progress curve (AUDPC). The spatial pattern of the disease was studied using interpolative geostatistical maps and by calculating dispersion indices (variance-to-mean ratio, Lloyd's index, and Morisita's index). At the subregional level, PLDS was characterized by applying multivariate analysis to a matrix comprising 10 orchards and 12 variables. Results showed that disease incidence ranged from 15% to 68%, with epidemic parameters also varying significantly among orchards ( $1/b = 0.000041 - 1.69$ ,  $p = 0.05$ ; AUDPC = 7.09 - 41.06;  $y_f = 15\% - 68\%$ ). In the 10 orchards evaluated, a predominantly random spatial pattern was observed ( $V/M = 0.7 - 1.1$ , LIP = 0.7 - 1.2 y  $I_g = 0.7 - 1.3$ ). The knowledge generated in this study may be useful for designing targeted

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management strategies for this abiotic disease, taking into account the sub-regional characteristics of the state of Veracruz.

## Keywords

*Citrus latifolia*, Abiotic Disease, Physiopathy, Epidemiology

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## 1. Introduction

Citrus cultivation is currently reported in 149 countries worldwide, with a total annual production of 277.8 million tonnes, primarily concentrated in regions with warm climates. The principal producers are China, Brazil, India, United States, and Mexico [1].

In Mexico, citrus production is distributed across 29 states, covering a cultivated area of 631,574.20 ha and generating an annual output of 9,094,525.18 tonnes. Persian lime (*Citrus latifolia* Tan.) is cultivated in 25 states, with Veracruz, Oaxaca, Yucatán, Jalisco, and Tabasco as the main producers. Veracruz stands out as the principal production zone, contributing 866,099.53 tonnes annually, equivalent to an estimated commercial value of 5492 million pesos [2].

Despite the economic importance of citrus production, its sustainability is increasingly threatened by certain physiological disorders [3]. One such disorder was first reported in Persian lime in Florida by Ruehl in 1943 [4], and later described in detail by Fawcett and Calavan in 1947 [5], who referred to it as “wood pocket”. Currently, it is considered an abiotic disease and, due to the wide range of symptoms observed in affected trees, it has been designated as Persian lime death syndrome (PLDS), known in Spanish as *síndrome de la muerte del limón Persa* (SMLP) [6].

This physiological disorder had been previously observed in the Martínez de la Torre region of Veracruz, although for several years it did not result in significant crop damage [7]. However, it has recently reemerged as a serious problem in local citrus production, causing the death of productive trees [8] [9]. This resurgence has been associated with high temperatures that cause tracheid rupture and xylem cavitation, leading to deterioration of the vascular system and tree death [10].

The disorder has also been detected in propagation material units, particularly in nurseries, budwood production lot, and foundation lot [11] [12]. In Persian lime trees, typical symptoms include asymmetric foliar chlorosis, xylem rot, sectorial fruit staining, abundant sucker growth on the rootstock, and tree death [6].

To date, studies on PLDS remain limited worldwide, and the available information does not provide a complete characterization of its causes. Therefore, the objective of this study was to provide a more precise characterization of this syndrome in Persian lime trees in the Martínez de la Torre region by tracking symptom progression in commercial orchards, to enhance early detection and support

field-level management strategies.

## 2. Materials and Methods

### 2.1. Location and Study Area

The study was carried out in commercial citrus orchards across the region comprising the municipalities of Martínez de la Torre, Atzalan, Tlapacoyan, San Rafael, and Nautla (**Figure 1**), where the presence of the syndrome had previously been confirmed.



**Figure 1.** Geographical location of the municipalities surveyed to evaluate the spatio-temporal incidence of Persian lime decline syndrome. This figure was created using MapChart “<https://www.mapchart.net> (accessed on 15 July 2025)”.

### 2.2. Selection of Study Sites

To evaluate the regional spread of the syndrome in the Martínez de la Torre area, collaboration was established with the State Committee for Plant Health to access the georeferenced database of Persian lime orchards located within the Phytosanitary Epidemiological Management Areas.

From this database, ten orchards with confirmed presence of the syndrome were randomly selected, based on their geographic representativeness and the prevalence of PLDS within the main citrus-growing region of Veracruz, an area where the disease has recently reemerged with greater incidence [6]. Within each orchard, a 10 × 10 tree plot (100 trees) was established, following the methodology of Domínguez-Monge *et al.* [13], ensuring that at least one tree exhibited visible symptoms associated with the syndrome.

The incidence of PLDS was estimated as the proportion of symptomatic trees within each plot. A tree was considered symptomatic if it showed one or more of the characteristic signs of the syndrome (as described in Section 2.5).

Incidence (%) was calculated as:

$$\text{Incidence (\%)} = \left( \frac{\text{ST}}{\text{TT}} \right) \times 100$$

where:

ST = Number of symptomatic trees.

TT = Total number of trees in the block.

Incidence values were calculated both per assessment (absolute incidence) and across time (cumulative incidence from the first to the final evaluation).

### 2.3. Temporal Progression of PLDS

Disease incidence was assessed biweekly within each selected plot from July to October 2024. Each tree was visually inspected for characteristic symptoms –primarily foliar chlorosis and sectorial fruit staining– to classify it as symptomatic or asymptomatic.

Temporal progression was evaluated using interpolated geostatistical maps and by modeling the incidence data with the Weibull distribution, following the methodology of Mora-Aguilera *et al.* [14]. The proportion of symptomatic trees per assessment was used as the dependent variable, while time (in biweekly intervals) was considered the independent variable.

The Weibull model was selected for in describing non-linear disease progress curves. Apparent infection rate ( $r$ ) was approximated as the inverse of parameter  $b$ , while the shape of the epidemic curve was defined by parameter  $c$ . Parameters were estimated using the PROC NLIN procedure in SAS software (version 9.0; SAS Institute Inc., Cary, NC, USA). This model provides advantages over linear alternatives particularly in capturing the asymmetrical progression patterns typical of plant disease epidemics [15]-[18].

Model fit was evaluated using the coefficient of determination ( $R^2$ ) between observed and predicted values. Residual analysis was also conducted to assess assumptions and detect potential deviations, as described by Campbell and Madden in 1990 [19].

Epidemic intensity was further quantified by calculating the Area Under the Disease Progress Curve (AUDPC), using trapezoidal integration [19].

### 2.4. Spatial Analysis of PLDS

As in the temporal analysis, disease incidence data at the tree level were used to assess spatial distribution patterns through the application of dispersion indices [20].

To assess the spatial spread of PLDS from July to October 2024, analyses were conducted based on the frequency distribution of symptomatic plants per sampling unit, with the aim of determining the degree of spatial association among diseased plants within orchards. As a first step, binary incidence data (presence or absence of disease) were used to generate dispersion maps using Surfer® 10 software.

Subsequently, spatial distribution was evaluated at each sampling date using three classical dispersion index: the Mean-Variance (V/M), Morisita's Index (I<sub>g</sub>), and Lloyd's Index of Patchiness (LIP), following the methodology described by Kranz (1993). The interpretation criteria for all three indices were as follows: values < 0.7 indicated a uniform distribution, values between 0.7 and 1.3 indicated a random distribution, and values > 1.3 indicated an aggregated distribution.

### 2.5. Symptom Characterization in Affected Trees

In the ten orchards where the presence of the syndrome was confirmed, symptom expression was characterized in each symptomatic tree to identify potential relationships among different symptom types. The following variables were recorder per tree: number of water sprouts, number of branch cracking, location (lower, meddle, upper canopy) and severity of foliar chlorosis (1 = 25%, 2 = 50%, 3 = 75%, 4 = 100%), severity of xylem rot (%), presence of branch wilting, and intensity of sectorial fruit staining (**Figure 2**). Evaluations were conducted biweekly over a four- month period.



**Figure 2.** Representative symptoms associated with Persian lime death syndrome. a) Typical foliar chlorosis, b) Sectorial fruit staining and c) Wood color change.

In addition to these variables, complementary orchard-level data were collected, including soil pH, temperature, and moisture, tree age, and rootstock type. These variables were compiled into a single dataset for multivariate classification analysis [21].

Data from commercial Persian lime orchards in the Martínez de la Torre region were analyzed using multivariate statistical techniques, including multivariate analysis of variance (MANOVA), principal component analysis (PCA), and factor analysis (FA), following the methodology of Domínguez-Monge *et al.* [22].

PCA and FA were conducted to identify the variables most strongly associated with disease expression across orchards. PCA was performed using the PROC

PRINCOMP procedure, while FA was conducted using PROC FACTOR in SAS software (version 9.0). Based on the most influential variables identified through FA, cluster analysis was performed using the average linkage method with the PROC CLUSTER procedure. This approach enabled the classification of orchards into distinct groups based on disease-related characteristics.

### 3. Results and Discussion

Persian lime trees were evaluated in ten commercial orchards located in the Martínez de la Torre region, Veracruz, where the presence of the syndrome had been previously confirmed. A systematic characterization of symptomatic expression and incidence was carried out in these orchards. Baseline incidence data recorded at the beginning of the evaluation period are summarized in **Table 1**.

**Table 1.** Characteristics of commercial Persian lime orchards and initial incidence of PLDS recorder during the 2024 survey in the Martínez de la Torre Region, Veracruz.

Orchard	Municipality	Area (ha)	Planting Density	Age	(+)	Latitude	Longitude	Alt	%	Root-stock	Sampling Date
JS-1	Tlapacoyan	5	6 × 4	3	3	20.0412	-97.1107	127	3%	C. v	30-jul-24
CM-2	Atzalan	3	3 × 3	3	20	20.0390	-97.05861	127	20%	C. a	15-ago-24
SR-3	Tlapacoyan	3	6 × 4	<3	29	20.0716	-97.1033	127	29%	C. v	15-ago-24
DJ-4	San Rafael	1	6 × 5	<2	50	20.2201	-96.86149	10	50%	C. a	15-ago-24
J-5	Nautla	18	6 × 3	<3	35	20.029	-96.90964	109	35%	C. v	15-ago-24
MG-6	Tlapacoyan	5	4 × 5	<4	20	20.0676	-97.10995	141	20%	C. v	15-ago-24
FT-7	Mtz de la Torre	3	4 × 5	7	18	20.039	-97.021	95	18%	C. a	30-ago-24
IA-8	Mtz de la Torre	5	5 × 3	7	25	20.1719	-97.04144	90	25%	C. a	30-ago-24
AH-9	Mtz de la Torre	5	6 × 4	3	11	20.0282	-97.03607	79	11%	C. v	30-ago-24
SL-10	Tlapacoyan	2	3 × 3	4	7	20.0778	-97.11379	126	7%	C. a	30-sep-24

(+) = Initial number of symptomatic trees; Alt = Altitude (m. a. s. l.); % = Initial incidence (%); C. v. = *Citrus volkameriana*; C. a. = *Citrus aurantium*.

#### 3.1. Temporal Analysis

##### 3.1.1. Progress Curve of PLDS

The incidence of PLDS in the ten commercial orchards evaluated increased substantially from the initial assessment in July through October 2024. Orchards J-5 and DJ-4 recorded the highest initial incidence rates, with 35% and 50%, respectively. In contrast, orchards JS-1, CM-2, SR-3, MG-6, FT-7, IA-8, AH-9, and SL-10 exhibited lower initial incidences, ranging from 3% to 29% (**Table 2**).

By October, final PLDS incidence ranged from 15% to 68% across orchards (**Figure 3**). Overall, disease spread progressed notably, reaching incidence levels comparable to those previously reported in young Persian lime orchards in the

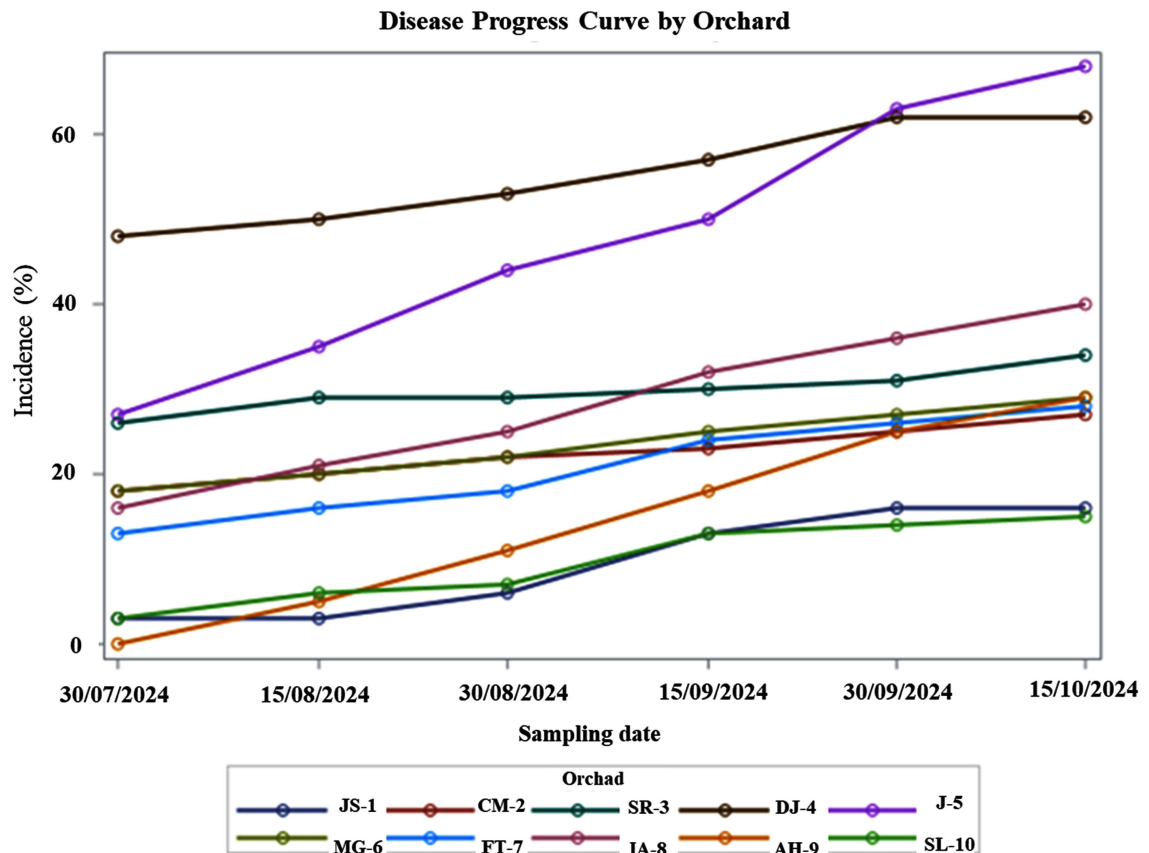
Martínez de la Torre region, where PLDS incidence reached up to 50% [23].

**Table 2.** Cumulative incidence of PLDS from July to October 2024 in ten commercial orchards surveyed in the Martínez de la Torre Region, Veracruz.

Orchard	Damage	Date 1	Date 2	Date 3	Date 4	Date 5	Date 6
		30/07/2024	15/08/2024	30/08/2024	15/09/2024	30/09/2024	15/10/2024
JS-1	(+)/Eval.	3	----	----	10	3	0
	Cum. Inc (%)	3	----	----	13	16	16
CM-2	(+)/Eval.	----	20	----	3	2	2
	Cum. Inc (%)	----	20	----	23	25	27
SR-3	(+)/Eval.	----	29	----	1	1	3
	Cum. Inc (%)	----	29	----	30	31	34
DJ-4	(+)/Eval.	----	50	----	7	5	0
	Cum. Inc (%)	----	50	----	57	62	62
J-5	(+)/Eval.	----	35	----	15	13	5
	Cum. Inc (%)	----	35	----	50	63	68
MG-6	(+)/Eval.	----	20	----	5	2	2
	Cum. Inc (%)	----	20	----	25	27	29
FT-7	(+)/Eval.	----	----	18	6	2	2
	Cum. Inc (%)	----	----	18	24	26	28
IA-8	(+)/Eval.	----	----	25	7	4	4
	Cum. Inc (%)	----	----	25	32	36	40
AH-9	(+)/Eval.	----	----	11	7	7	4
	Cum. Inc (%)	----	----	11	18	25	29
SL-10	(+)/Eval.	----	----	7	6	1	1
	Cum. Inc (%)	----	----	7	13	14	15

(+) Number of Positive Trees.

Two distinct within-orchard spread patterns were identified based on progress curves: 1) high-spread orchards (J-5 and DJ-4), with final incidence ranging from 62% to 68%; and 2) low-spread orchards (JS-1, CM-2, SR-3, MG-6, FT-7, IA-8, AH-9, and SL-10), with values between 15% and 40% (Figure 3). Although epidemic intensities differed, PLDS occurrence in both groups may be associated with the origin of the grafting material or with environmental stress factors, particularly high temperatures and severe water stress, as suggested by previous studies [24].



**Figure 3.** Temporal progression of PLDS incidence in 10 commercial Persian lime orchards in the Martínez de la Torre region, Veracruz, based on biweekly assessment conducted from July to October 2024.

### 3.1.2. Temporal Models of PLDS Progression

Using cumulative incidence data, the parameters of the Weibull model were fitted for all orchards at each assessment date. The progression of the epidemics was adequately described by this flexible model, with coefficients of determination ( $R^2$ ) ranging from 0.67 to 0.99 (Table 3).

Given the abiotic nature of the syndrome—where no infectious (contagious) agent is involved—the apparent progression rates ( $1/b$ ) estimated in this study are more appropriately interpreted as rates of plant damage rather than true infection. These rates did not consistently align with the temporal progression of the syndrome across orchards, as indicated by a moderate Pearson correlation coefficient ( $r = 0.56$ ). Notably, only orchards J-5 and DJ-4, which showed the highest final incidences (68% and 62%, respectively), also exhibited the highest apparent progression rates ( $1/b$ ), with  $b = 0.06$  and  $b = 1.69$ , respectively. No consistent correlation was observed in the remaining orchards (Table 3).

As a complementary indicator of PDLIS intensity in the Martínez de la Torre region, Veracruz, the area under the disease progress curve (AUDPC) was also calculated. Unlike the rate parameter  $b$ , AUDPC values were strongly correlated with the temporal progression by orchard ( $r = 0.96$ ). The highest AUDPC values

were observed in orchards J-5 (35.615) and DJ-4 (41.060), which also presented the highest final incidences (68% and 62%, respectively). In contrast, orchards JS-1 (7.095) and SL-10 (7.150), which had the lowest final incidences (16% and 15%, respectively), recorded the lowest AUDPC values (Table 3).

**Table 3.** Estimated parameters of the Weibull model describing PLDS progress curves in 10 commercial Persian lime orchards in the Martínez de la Torre region, Veracruz, based on cumulative incidence data collected from July to October 2024.

Orchard	Weibull Model <sup>a</sup>				AUDPC	Y <sub>0</sub>	Y <sub>f</sub>
	$y = 1 - e^{-(t/b)^c}$	<i>c</i>	1/ <i>b</i>	R <sup>2</sup>			
JS-1	$y = 1 - e^{-(t/262.7)^{0.991}}$	0.991	0.0038066	0.91	7.095	0.03	0.16
CM-2	$y = 1 - e^{-(t/24114.4)^{0.136}}$	0.136	0.0000415	0.81	16.685	0.18	0.27
SR-3	$y = 1 - e^{-(t/20094.1)^{0.085}}$	0.085	0.0000498	0.76	22.075	0.26	0.34
DJ-4	$y = 1 - e^{-(t/0.5902)^{0.126}}$	0.126	1.6943409	0.67	41.060	0.48	0.62
J-5	$y = 1 - e^{-(t/16.4756)^{0.385}}$	0.385	0.0606958	0.76	35.615	0.27	0.68
MG-6	$y = 1 - e^{-(t/14761.3)^{0.138}}$	0.138	0.0000677	0.78	17.435	0.18	0.29
FT-7	$y = 1 - e^{-(t/1665.1)^{0.249}}$	0.249	0.0006006	0.81	15.530	0.13	0.28
IA-8	$y = 1 - e^{-(t/200.2)^{0.321}}$	0.321	0.0049950	0.84	21.115	0.16	0.40
AH-9	$y = 1 - e^{-(t/121.3)^{1.232}}$	1.232	0.0082440	0.99	11.000	0.00	0.29
SL-10	$y = 1 - e^{-(t/817.4)^{0.589}}$	0.589	0.0012234	0.91	7.150	0.03	0.15

Model parameters were estimated by nonlinear regression based on the Weibull model. In the equation, *c* represents the shape parameter, *b* the rate parameter, *y* the abiotic disease progression measured as the incidence of symptomatic trees, and *t* time (in years). *y*<sub>0</sub> denotes the initial incidence proportion, and *y*<sub>f</sub> the final incidence proportion of PLDS in the evaluated Persian lime (*Citrus latifolia*) orchards in the Martínez de la Torre region, Veracruz.

## 3.2. Spatial Analysis

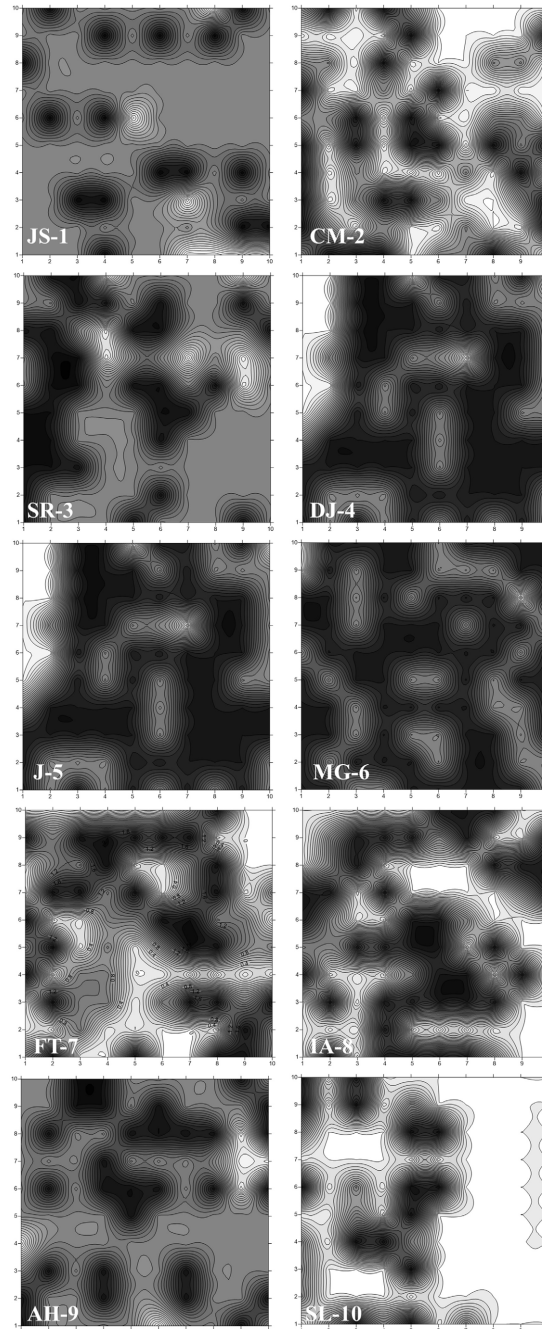
### 3.2.1. Geostatistical Mapping of PLDS Distribution

Geostatistical contour maps revealed spatial patterns in the distribution of PLDS across all evaluation periods (Figure 4). Symptomatic trees were detected both within individual rows and between adjacent rows. The 10 × 10 plot mapping enabled efficient visualization of the spatial distribution of symptomatic and asymptomatic trees, providing a useful preliminary diagnostic tool. This spatial mapping was further supported and refined through the quantitative analysis of dispersion indices, as discussed in subsequent sections.

### 3.2.2. Spatial Distribution Pattern of PLDS in Persian Lime Orchards

The dispersion indices, calculated using quadrants of four trees, revealed a predominantly random spatial distribution of PLDS-symptomatic trees across all evaluated orchards, with the exception of CM-2 (Table 4). According to Campbell and Madden [19], plant spatial patterns result from the interaction of multiple factors. In this case, involving a non-infectious abiotic syndrome, the symptom dynamics showed a clearly random pattern. This finding supports the hypothesis

that the spread of PLDS is passive in nature, likely associated with the use of symptomatic propagative material (e.g., affected buds) and/or environmental stressors, particularly high regional temperatures [25]. These stress conditions may accelerate the onset and expression of symptoms, as previously noted by Roistacher [26].



**Figure 4.** Spatial distribution of PLDS in 10 commercial Persian lime orchards in the Martínez de la Torre region, Veracruz, based on incidence data collected from July to October 2024. Geostatistical contour maps were used to visualize the disease pattern within each orchard. Darker areas represent zones with higher concentrations of symptomatic trees.

**Table 4.** Spatial distribution pattern of PLDS in 10 commercial Persian lime orchards in the Martínez de la Torre region, Veracruz.

Orchard	Final Incidence (%)	V/M	Pattern	LIP	Pattern	Ig	Pattern
JS-1	16	0.7	random	0.6	uniform	0.6	uniform
CM-2	27	0.5	uniform	0.6	uniform	0.6	uniform
SR-3	34	1.1	random	1	random	1.1	random
DJ-4	62	0.7	random	0.9	random	0.9	random
J-5	68	0.3	uniform	0.7	random	0.7	random
MG-6	29	0.8	random	0.9	random	0.9	random
FT-7	28	0.7	random	0.7	random	0.8	random
IA-8	40	0.7	random	0.8	random	0.9	random
AH-9	29	0.8	random	0.8	random	0.8	random
SL-10	15	1.1	random	1.2	random	1.3	random

V/M = Mean-Variance Index; LIP = Lloyd's Index of Patchiness; Ig = Morisita's Index (Kranz, 1993).

### 3.2.3. Multivariate Analyses of PLDS

The statistical analysis identified three principal components based on the eigenvalues, collectively explaining 71% of the total variance. Three variables were selected according to their individual eigenvalues ( $\geq 1.93$ ) and their cumulative contribution to the explained variance (Table 5). Other soil-related variables, such as pH, temperature, and moisture were also included in the analysis; however, they did not significantly contribute to the characterization of the principal components.

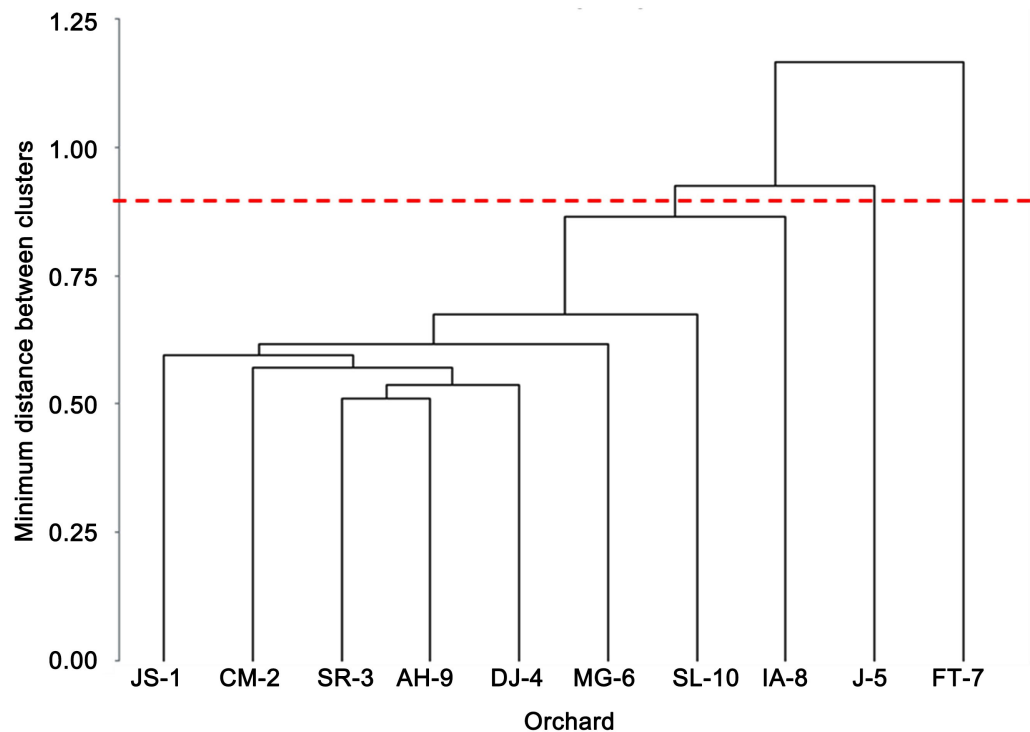
**Table 5.** Principal components, corresponding eigenvalues from the correlation matrix, and selected contributing variables.

Component	Eigenvalue	Difference	Proportion	Cumulative	Variable
1	3.6	1.28	0.32	0.32	Age
2	2.32	0.38	0.21	0.53	Branch wilting
3	1.93	0.52	0.17	0.71	Foliar chlorosis severity

### Formation of Risk Groups for PLDS

Using the three most influential variables identified in the PCA—tree age, number of wilted branches, and foliar chlorosis severity—a hierarchical cluster analysis was performed. By applying a dendrogram cut at a Euclidean distance of 0.90, distinct orchard groups were formed based on their susceptibility to the syndrome. The groups were defined as follows: Group 1 included orchard H7, characterized by mature trees ( $>7$  years), low number of wilted branches, and high severity of typical foliar chlorosis; Group 2 comprised orchard H5, with young trees ( $<3$  years), a high number of wilted branches, and moderately high foliar chlorosis severity; Group 3 consisted of orchards JS-1, CM-2, SR-3, AH-4, MG-6, IA-8, J-5, and FT-7, all with young trees, a moderate number of wilted branches,

and low foliar chlorosis severity in the canopy (Figure 5).



**Figure 5.** Cluster dendrogram showing the grouping of 10 commercial orchards affected by PLDS, based on a Euclidean distance threshold of 0.90. Clustering was performed using three key inductive variables: tree age, branch wilting, and foliar chlorosis severity. The dashed line indicates the cut-off point used to define the number of clusters.

#### 4. Conclusions

During the four-month study period (July to October 2024), the incidence of trees exhibiting PLDS symptoms increased significantly, reaching up to 68% in certain orchards. The epidemic parameters varied notably among the ten commercial orchards assessed in the Martínez de la Torre region, with apparent damage rates ( $1/b$ ) ranging from 0.000041 to 1.69 ( $p = 0.05$ ), final incidence values ( $y_f$ ) between 15% and 68%, and AUDPC values from 7.09 to 41.06.

The spatial distribution of symptomatic trees was predominantly random in most orchards, as indicated by dispersion indices ( $V/M = 0.7 - 1.1$ ,  $LIP = 0.7 - 1.2$ , and  $I_g = 0.7 - 1.3$ ). This spatial pattern suggests that PLDS is likely associated with non-contagious factors, such as the use of symptomatic propagative material and/or exposure to environmental stressors, particularly elevated regional temperatures and water scarcity.

Multivariate analysis based on tree age, number of wilted branches, and foliar chlorosis severity enabled the classification of the orchards into three distinct clusters at a Euclidean distance of 0.90. The largest group, comprising eight orchards, was characterized by young trees, a moderate number of wilted branches, and low chlorosis severity, and was associated with a higher potential for PLDS develop-

ment.

Our findings demonstrate that the spatial and temporal dynamics of PLDS in Persian lime-growing area of Martínez de la Torre can be effectively characterized using geostatistical dispersion indices and multivariate analysis methods. These approaches enabled the identification of spatial randomness, risk-associated orchard typologies, and the potential influence of abiotic factors on syndrome expression.

Given the predominantly random spatial distribution of symptomatic trees and the apparent association with environmental stress and grafting material, effective management of PLDS should involve integrated approaches emphasizing epidemiological monitoring, the use of certified propagative material, and early identification of risk conditions conducive to symptom expression.

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### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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