Anais da Academia Brasileira de Ciências (2013) 85(4): 1567-1576
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-37652013112412
www.scielo.br/aabc

Spatial and temporal analysis of stem bleeding disease in coconut palm in the state of sergipe, Brazil

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Manuscript received on September 3, 2012; accepted for publication on April 15, 2013

ABSTRACT

Stem bleeding disease (resinosis) of coconut palm is caused by *Thielaviopsis paradoxa* and is very important in the state of Sergipe, Brazil. Understanding the epidemiological behavior of the disease is essential for establishing more efficient control strategies. Thus, we characterized the temporal progression and spatial distribution of stem bleeding in a commercial orchard under conditions of natural infection in the area of Neopolis, Sergipe. Three plots with 729 plants each were selected and evaluated every two months for stem bleeding incidence. In the temporal analysis, the monomolecular model gave the best fit to data on disease incidence, as it accurately showed the temporal dynamics of the disease during the experiment period. The spatial pattern of stem bleeding varied over time, with initial infections presenting random pattern and then evolving to aggregate pattern during evaluations. This indicates that the disease may have originated from the pathogen survival structures, followed by auto infections caused by dissemination from plant to plant, either by humans, by contact between roots, or by the vector *Rhynchophorus palmarum*.

Key words: coconut, epidemiology, stem bleeding, *Thielaviopsis paradoxa*.

INTRODUCTION

Coconut palm (*Cocos nucifera* L.) is one of the most important trees in the world, as it generates employment and income in many countries where its fruit is either eaten raw or processed into manufactured products and by-products. However, many factors such as diseases can reduce crop yield.

Coconut stem bleeding, caused by the fungus *Thielaviopsis paradoxa* (De Seynes) Höhn, is an important disease for coconut crops. It was first detected in the state of Sergipe in early 2004 and has then become the main concern of producers, researchers, and extension agents due to its rapid spread and subsequent death of infected plants in less than two months. Aggravating the situation, there is strong evidence that the beetle *Rhynchophorus palmarum* Linnaeus performs as one of the disease vectors in the field (Costa-Carvalho et al. 2011).

The main stem bleeding symptoms are reddish-brown liquid in the stem cracks, which may turn

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blackish when dried; reduced frequency of leaf emergence; reduced size of young leaves; stem thinning near the canopy as disease progresses; brownish-yellow and easily breakable leaves (Warwick and Passos 2009).

Information about spatial and temporal dynamics of stem bleeding is extremely important to better understand the pathosystem, as it can be useful to describe and clarify the disease progression. In addition, this information helps develop sampling plans, plan controlled experiments, and characterize production losses caused by the disease.

Temporal analysis allows building disease progress curves for better representation of the epidemic process. Interpreting these curve shapes and determining their components, such as initial amount of disease, progression rate, final amount of disease, and area under the progress curve are fundamental to manage epidemics (Bergamin Filho 1995).

The study of spatial patterns of plant diseases, which reflects the dispersion process of pathogens characterized by the distribution of sick plants relative to each other, provides helpful data to elucidate the etiology, the role of dissemination agents such as vectors, wind and rain, and to define the most appropriate strategies for disease control. The analysis of spatial arrangement also enables better understanding of the dynamics of epidemics in relation to the pattern of the initial inoculum, the effect of biological and cultural practices, and the effect of environmental factors on the amount of disease in the field (Jeger 1990).

Thus, we characterized the spatial and temporal dynamics of stem bleeding in the main coconut producing region of the state of Sergipe by creating disease progress curves. In addition, we characterized the structure and dynamics of stem bleeding outbreaks and analyzed ordinary runs.

MATERIALS AND METHODS

The experiments were conducted at the Laboratory of Phytopathology of Embrapa Tabuleiros Costeiros, Aracaju, and at the União Fruticultura Farm located in Perímetro Irrigado de Neópolis, state of Sergipe, latitude 10°17'S, longitude 36°35'W, and altitude 120 m. This property was chosen because it had the first outbreaks in 2009, nearly five years after disease detection in other local plantations.

Temperature data, relative humidity, and total rainfall were obtained daily at the weather station of the Associação dos Concessionários do Distrito de Irrigação do Platô de Neópolis- ASCONDIR, located about 5 km from the experiment areas.

Evaluation of Disease

We selected three homogeneous plots comprising 13-year-old green dwarf coconut palms with 729 plants each. The trees were distributed in 27 rows, 27 columns and planted in equilateral triangle system (7.5 m side). Disease incidence was assessed every two months during two years, from July 2009 to May 2011, starting from the earliest plants showing stem bleeding symptoms.

All plants in every plot were monitored individually for typical stem bleeding symptoms. We recorded the presence or absence of symptoms, i.e., incidence, and the relative position of each plant to create a map per plot for each evaluation date. Based on these maps we have obtained cumulative incidence maps, which consider that sick plants will remain sick in later assessments.

Disease Progress Curves

The progress curves were designed for all plots with values of incidence relative to time. The incidence was determined by the percentage of plants showing stem bleeding symptoms.

Based on the incidence values in proportion (y), we have adjusted the following models: Linear ($y = y_0 + r * t$); Exponential: $\exp(x_0) * \exp(rt)$; Monomolecular: $1 - ((1 - \exp(x_0)) / (\exp(x_0))) * \exp(-rt)$; Logistics: $1 / (1 + ((1/\exp(x_0)) - 1) * \exp(-rt)$; and Gompertz: $\exp(-(-\ln(\exp(-\exp(x0)))) * \exp(-rt))$. The best-fit model was selected taking into account the highest coefficient of determination adjusted in the regression analysis (*R2*) for reciprocity between the observed values (independent variable) and predicted values (dependent variable) of disease incidence; the least mean square (LMS); and the absence of trends in the residual plot (Campbell and Madden 1990).

Based on the best-fit model, we determined the linearization to the curve. Then we estimated the onset of the epidemic in each plot and the amount of disease at 660 days.

Spatial Arrangement of the Disease

Analysis of doublet

The numbers of healthy (•) and sick (x) plants were characterized in the crop rows. The expected number of doublets was calculated under the null hypothesis E (D) = m (m-1) / N, where D: number of doublets, m: number of sick plants, N: number of plants in the row. The standard deviation of D under the null hypothesis was calculated by S (D) = $\{m(m-1)[N(N-1)+(2N(m-2)+N(m-2)(m-3)-(N-1)m(m-1)]/N^2(N-1)\}^{0.5}$. We calculated the standardized value of ZD = $[(D+0.5-E(D)]/s(D)$ based on normal distribution. When ZD> 1.64 (P = 0.05), it was defined as aggregate pattern, and when ZD <1.64 (P = 0.05) it was deemed random pattern.

Analysis of dynamics and structure of outbreaks (ADEF)

Outbreak sources were deemed areas of localized concentration of sick plants, either as primary infection sources or coincident with areas originally favorable to disease development, which tend to influence the later pattern of transmission (Laranjeira et al. 1998).

Only sick plants immediately adjacent in the pattern of vertical, horizontal, or longitudinal closeness shared the same outbreak source. In each plot we counted the number of outbreak sources (NF), number of plants per outbreak source (NPF), number of sick plants in the largest row (IF), and number of sick plants in the largest column (Ic) in each outbreak source. Based on this data we calculated the average number of plants per outbreak source (NMPF), frequency distribution of number of plants per outbreak source (NPF), outbreak shapes (IFF), and compression ratio per outbreak (ICF) using the formulas: IFF= If / Ic and ICF= NPF / If* Ic.

RESULTS AND DISCUSSION

Average values of temperature, relative humidity, and total rainfall during the experiments were respectively 26°C, 91 mm, and 81.4%. The highest temperatures were recorded in March in both years (approximately 28°C). Rainfall rates showed significant differences during the experiment period. The highest values were obtained in July 2009 and July 2010, 177.3 and 214.4 mm respectively. Relative humidity ranged from 74.8 to 89.6% (Figure 1).

Disease Progress Curves

In all three plots, disease progressed intermittently over the two years with increased incidence of stem bleeding. At the end of the experiment, the highest value of incidence was found in plot 3, which had 22.77% plants with disease symptoms, followed by plot 1 and 2, with 20.16 and 12.89% respectively (Figure 2).

Taking into account the coefficient of determination adjusted in the regression analysis (R2), the mean square deviations (Table I) and the residual chart, the monomolecular model best fit the data on stem bleeding incidence among the plots evaluated in the study period. Disease progression rates (r) estimated by the parameter b in the regression equation were 0.0003, 0.0002, and 0.0004 in plots 1, 2 and 3 respectively (Figure 2).

In the two-year evaluation, the period from May to September showed the highest rates of rainfall, relative humidity, and lower temperatures rates.

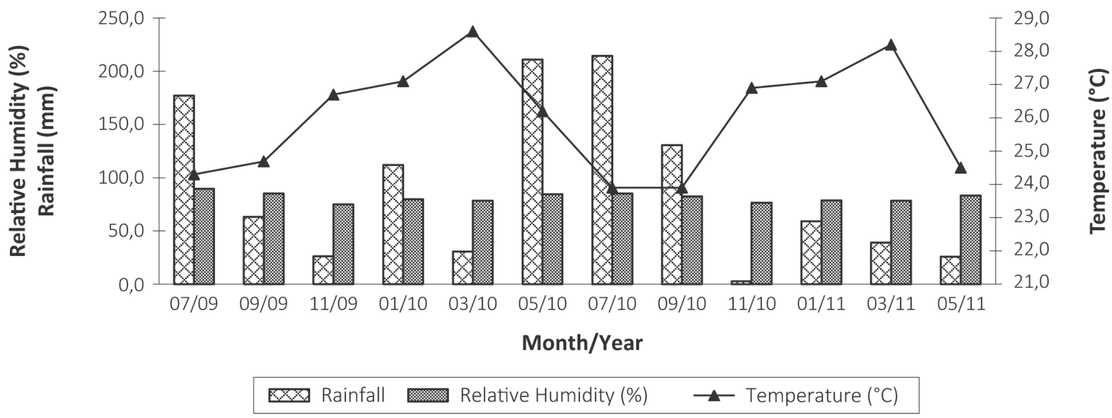

Figure 1 - Average temperature, total rainfall, and relative humidity in Platô de Neópolis, state of Sergipe, from July 2009 to May 2011.

However, disease progression was gradual and constant, and was not influenced by the time of year (Figure 2). This result indicates the great adaptability of pathogens to different environmental conditions, as well as the possibility of involvement of other factors for the occurrence of disease. The lack of correlation between incidence of stem bleeding and climate variables in all plots shows that climatic conditions do not affect disease progress in the case of coconut palms.

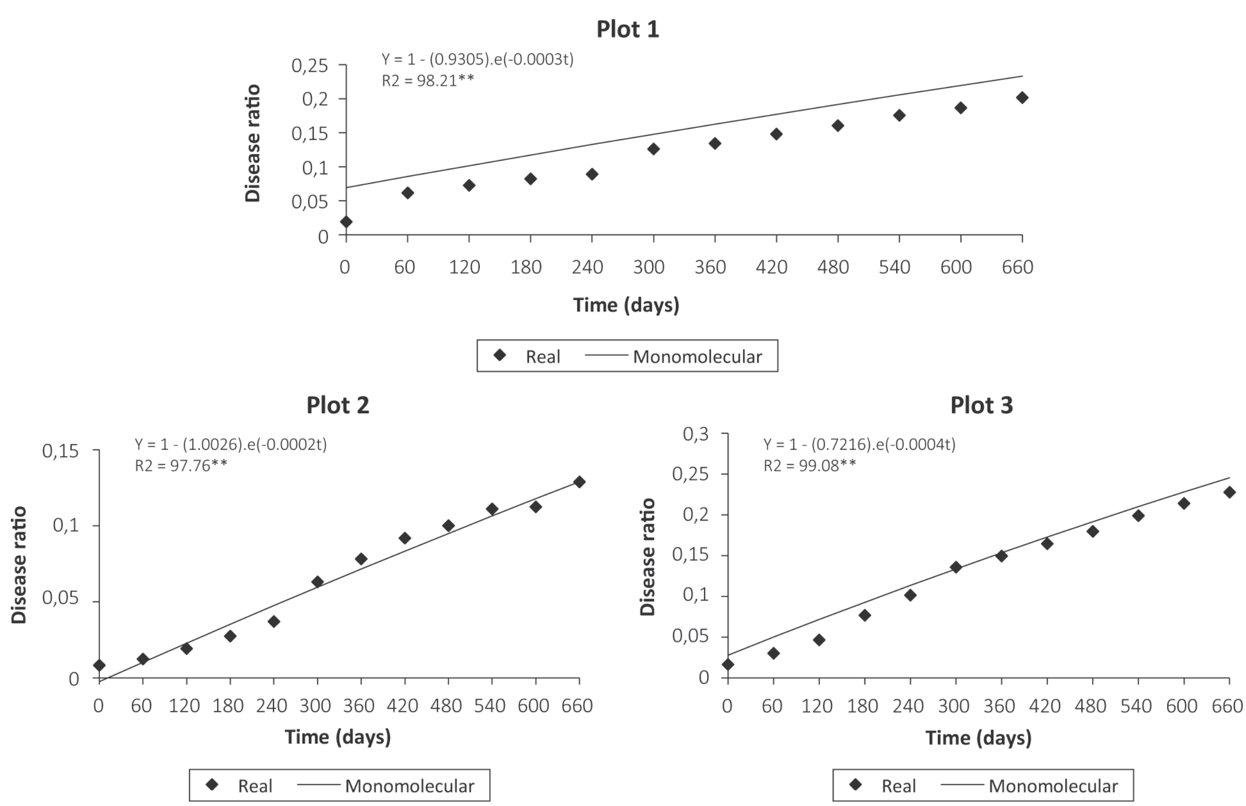

Figure 2 - Progress curves of incidence of coconut stem bleeding in Platô de Neópolis, state of Sergipe, in plots 1, 2 and 3, adjusted to a monomolecular model.

TABLE I
Coefficient of determination between predicted and observed values (R^2) and residual mean square (*RMS*) of models for incidence of coconut stem bleeding in Platô de Neópolis, state of Sergipe.

Models	Plot 1		Plot 2		Plot 3	
	R^2	*RMS*	R^2	*RMS*	R^2	*RMS*
Linear	97.88	7.42x10-5	97.67	4.87x10-5	98.61	8.19x10-5
Monomolecular	98.21	6.27x10-5	97.76	4.93x10-5	99.08	5.42x10-5
Logistic	91.08	3.12x10-4	86.29	2.86x10-4	87.78	7.19x10-4
Exponential	89.05	3.83x10-4	84.01	3.34x10-4	84.01	9.41x10-4
Gompertz	94.69	1.86x10-4	92.84	1.5x10-4	93.75	3.68x10-4

R^2: coefficient of determination between predicted and observed values; RMS: residual mean square.

Spatial Arrangement of Disease

Analysis of doublet

By the analysis of doublet we found an aggregated spatial arrangement ($Z \geq 1.64$; $P = 0.05$) of sick plants starting from the second evaluation in 100% rows of all three plots (Table II).

Analysis of dynamics and structure of outbreaks (ADEF)

Plants with stem bleeding symptoms immediately adjacent in the pattern of vertical, horizontal or diagonal closeness were considered outbreak sources (Bergamin Filho et al. 2004).

The percentages of sick plants (PPD) corresponding to plots 1, 2 and 3 were 1.92, 0.82, and 1.65% respectively in the first assessment, and 20.16, 12.89, and 22.77% in the last evaluation after two years (Table III). The highest number of outbreaks per unit (NFU) was observed in the first evaluations in all plots. As PPD increased, NFU reduced and the mean number of plants per outbreak (NMPF) increased. This fact indicates that, in general, the epidemic started in isolated plants, which acted as inoculum sources to adjacent healthy plants.

A study on citrus sudden death has shown that in plots with up to 2% symptomatic plants, 85% outbreaks developed from a single plant, while in cases with up to 10% incidence over 65% were single outbreaks (Gottwald et al. 1996). According to the authors, epidemic onset of citrus sudden death occurs through isolated plants, which was also reported for citrus variegated chlorosis (Laranjeira et al. 1998, Nelson 1996, Nunes et al. 2001). This positive relationship between aggregation and incidence of symptomatic plants is consistent with the hypothetical behavior of vectors from external sources, which introduce the infectious disease in the plot. As vectors reach the area they spread through trees, which initially show a typically random spatial pattern of symptomatic plants. As vectors move inside the plot, especially through adjacent or neighboring trees, the aggregation of symptomatic plants increases (Jesus Junior et al. 2004). Thus, the beetle *Rhynchophorus palmarum* could be acting as a stem bleeding vector and spreading the disease through the field, which results in aggregation of sick plants near the outbreak source.

In the case of blister spot, the authors found a total of 10 outbreaks with average 2.5 plants/outbreak, and high number of single outbreaks representing 52% sick plants (Ferreira et al. 2009). Blister spot of coffee may be spread by sick seedlings that become adult plants in the field (Ferreira et al. 2009).

Analysis of outbreak shapes (IFF) in outbreak sources formed by a single plant showed IFF equal to 1.0, indicating isodiametric outbreaks. In other outbreaks and evaluations, IFF was greater than 1.0, indicating longer outbreaks between crop rows (Table III).

TABLE II
Spatial arrangement of coconut stem bleeding analyzed by doublet test in Platô de Neópolis, state of Sergipe, from July 2009 to May 2011.

Evaluation (month/year)	Plot	D	m	E(D)	S(D)	Z(D)	Spatial Pattern
jul/09	1	0	14	0.250	0.241	0.510	Random
	2	0	6	0.041	0.041	2.278	Aggregate
	3	0	12	0.181	0.176	0.761	Random
sep/09	1	23	45	2.716	2.398	13.422	Aggregate
	2	2	9	0.099	0.097	7.726	Aggregate
	3	8	22	0.634	0.598	10.175	Aggregate
nov/09	1	31	53	3.781	3.260	15.352	Aggregate
	2	6	14	0.250	0.241	12.737	Aggregate
	3	18	34	1.539	1.403	14.320	Aggregate
jan/10	1	39	60	4.856	4.101	17.107	Aggregate
	2	12	20	0.521	0.494	17.036	Aggregate
	3	37	56	4.225	3.611	17.511	Aggregate
mar/10	1	43	65	5.706	4.748	17.345	Aggregate
	2	16	27	0.963	0.895	16.419	Aggregate
	3	42	74	7.410	5.999	14.326	Aggregate
may/10	1	67	92	11.484	8.794	18.889	Aggregate
	2	30	46	2.840	2.500	17.496	Aggregate
	3	61	99	13.309	9.969	15.263	Aggregate
jul/10	1	68	98	13.040	9.798	17.718	Aggregate
	2	33	57	4.379	3.731	15.076	Aggregate
	3	68	109	16.148	11.715	15.295	Aggregate
sep/10	1	77	108	15.852	11.537	18.150	Aggregate
	2	37	67	6.066	5.017	14.035	Aggregate
	3	74	120	19.588	13.712	14.829	Aggregate
nov/10	1	84	117	18.617	13.160	18.161	Aggregate
	2	38	73	7.210	5.855	12.931	Aggregate
	3	83	131	23.361	15.767	15.145	Aggregate
jan/11	1	89	128	22.299	15.202	17.236	Aggregate
	2	41	81	8.889	7.044	12.287	Aggregate
	3	93	145	28.642	18.438	15.105	Aggregate
mar/11	1	92	136	25.185	16.716	16.464	Aggregate
	2	45	82	9.111	7.198	13.564	Aggregate
	3	100	156	33.169	20.556	14.851	Aggregate
may/11	1	100	147	29.440	18.822	16.379	Aggregate
	2	50	94	11.992	9.125	12.748	Aggregate
	3	105	166	37.572	22.480	14.327	Aggregate

m: number of infected plants; D: number of doublets; E(D): expected doublets; S(D): standard deviation, Z(D): doublet pattern normal distribution.

TABLE III
Analysis of dynamics and structure of outbreak sources (ADEF) in three plots of dwarf coconut palms with 729 trees each.

Evaluation	Plot	PPD	NF	NFU	NMPF	If	Ic	IFF	ICF
jul/09	1	1.92	13	12	1.08	1.00	1.00	1.00	1.08
	2	0.82	5	4	1.20	1.20	1.00	1.20	1.00
	3	1.65	12	12	1.00	1.00	1.00	1.00	1.00
sep/09	1	6.17	11	5	4.09	2.09	1.95	1.07	1.00
	2	1.23	6	4	1.50	1.33	1.00	1.50	1.50
	3	3.02	11	2	2.00	1.73	1.00	1.73	1.16
nov/09	1	7.27	11	5	4.82	2.55	1.45	1.76	1.30
	2	1.92	5	3	2.80	1.60	1.20	1.33	1.46
	3	4.66	9	1	3.78	2.67	1.22	2.19	1.16
jan/10	1	8.23	10	3	6.00	2.90	1.50	1.93	1.38
	2	2.74	5	0	4.00	2.40	1.20	2.00	1.39
	3	7.68	10	2	5.60	3.80	1.20	3.17	1.23
mar/10	1	8.92	9	3	7.22	3.33	1.67	1.99	1.30
	2	3.70	5	0	5.40	3.20	1.40	2.29	1.21
	3	10.15	9	0	8.22	4.87	1.67	2.92	1.01
may/10	1	12.62	5	0	18.40	5.20	2.20	2.36	1.61
	2	6.31	7	0	6.57	3.86	1.43	2.70	1.19
	3	13.58	10	1	9.90	5.10	1.90	2.68	1.02
jul/10	1	13.44	7	3	14.00	5.00	2.43	2.06	1.15
	2	7.82	7	0	8.14	4.14	1.71	2.42	1.15
	3	14.95	10	0	10.90	5.10	2.10	2.43	1.02
sep/10	1	14.81	8	1	13.50	4.13	2.40	1.72	1.36
	2	9.19	10	3	6.70	3.40	1.70	2.00	1.16
	3	16.46	11	1	10.90	4.82	2.00	2.41	1.13
nov/10	1	16.05	7	0	16.71	5.14	2.57	2.00	1.26
	2	10.01	9	1	8.11	3.67	1.78	2.06	1.24
	3	17.97	10	0	13.10	5.70	2.30	2.48	1.00
jan/11	1	17.56	7	0	18.29	4.29	3.14	1.37	1.36
	2	11.11	9	0	9.00	4.11	2.11	1.95	1.04
	3	19.89	10	0	14.50	6.20	2.40	2.58	0.97
mar/11	1	18.66	8	1	17.00	4.88	2.88	1.69	1.21
	2	11.25	8	1	10.25	3.88	2.38	1.63	1.11
	3	21.40	10	0	15.60	6.60	2.50	2.64	0.95
may/11	1	20.16	6	0	24.50	6.67	3.17	2.10	1.16
	2	12.89	8	0	11.75	3.88	2.50	1.55	1.21
	3	22.77	12	0	13.83	5.92	2.42	2.45	0.97

PPD: percentage of sick plants; NF: number of outbreaks; NFU: number of single outbreaks; NMPF: average number of plants per outbreak; Ic: number of sick plants in the largest column; If: number of sick plants in the longest row; IFF: outbreak shapes; ICF: compression ratio of outbreaks.

Citrus sudden death showed trends of IFF (outbreak shapes) values near 1.0, especially in cases of high number of single outbreaks (Jesus Junior et al. 2004). In the case of citrus variegated chlorosis (CVC), most plants in crop rows showed elliptical shape (Laranjeira et al. 1998, Nelson 1996). Likewise, (Ferreira et al. 2009, Jesus Júnior and Bassanezi 2004) elliptical shape was found in studies on the structure and dynamics of outbreaks for citrus sudden death (CSD) and blister spot respectively. Many authors correlate the spread of disease within crop rows to the closeness of plants as compared to the distance between rows (Laranjeira et al. 1998, Nelson 1996, Jesus Júnior and Bassanezi 2004, Ferreira et al. 2009). As in this case the distance between coconut palms within the row was equal to the distance between rows, cultural practices along crop rows, such as pruning, fruit harvest, and application of pesticides are not primarily responsible for the spread of disease in the field, which makes a vector involvement more evident in the process.

More compact outbreaks show ICF (compression ratio per outbreak) values close to 1.0 whereas less compact ones have lower values (Nelson 1996). In our study, increased NMPF (average number of plants per outbreak source) and decreased ICF (compression ratio per outbreak) remaining close to 1.0 during evaluations indicate compact outbreaks and aggregation of plants belonging to the outbreak sources (Table 3). This aggregation of coconut palms with stem bleeding symptoms corroborates the idea that the primary mode of transmission is via insect vector. Similar results were found for citrus sudden death (CSD) and citrus variegated chlorosis (CVC) (Laranjeira et al. 1998, Jesus Junior et al. 2004).

The dynamics and structure of stem bleeding outbreak sources showed similarity to other biotic diseases spread by vectors (Gottwald et al. 1989, 1995, 1996, 1998, Van de Lande 1993, Jeger 1990, Laranjeira et al. 1998, Jesus Junior et al. 2004).

In this study, the presence of multiple outbreaks within plots may be related to survival chlamydospores produced by the pathogens.

Stem bleeding was responsible for large aggregations of sick plants (Figure 3), indicating that infected plants were used as outbreak sources for subsequent infections (auto infections spread from plant to plant). This fact also showed that secondary inoculants were spread over short distances either by vectors, human handling of sick and then healthy plants, or contact between contaminated adjacent roots.

RESUMO

A resinose do coqueiro, causada por *Thielaviopsis paradoxa*, constitui uma importante doença do coqueiro no estado de Sergipe. A compreensão do comportamento epidemiológico da resinose é fundamental para o estabelecimento de estratégias de controle mais eficientes. Neste contexto o presente trabalho teve como objetivo caracterizar o progresso temporal e a distribuição espacial da resinose em um pomar comercial sob condições naturais de infecção de Neópolis, Sergipe. Foram selecionados 3 talhões com 729 plantas cada, onde as plantas foram avaliadas a cada dois meses quanto à incidência da doença. Na análise temporal, o modelo Monomolecular foi o que melhor se ajustou aos dados de incidência da resinose, representando bem a dinâmica temporal da doença durante o período experimental. O padrão espacial da resinose variou no decorrer do tempo, com infecções iniciais apresentando padrão aleatório, evoluindo para agregado no decorrer das avaliações, indicando que a doença pode ter sido originada de estruturas de sobrevivência no patógeno, seguidas de autoinfecções decorrentes da disseminação planta-a-planta, seja pelo homem, pelo contato entre raízes ou pelo vetor *Rhynchophorus palmarum*.

Palavras-chave: coqueiro, epidemiologia, resinose, *Thielaviopsis paradoxa*.

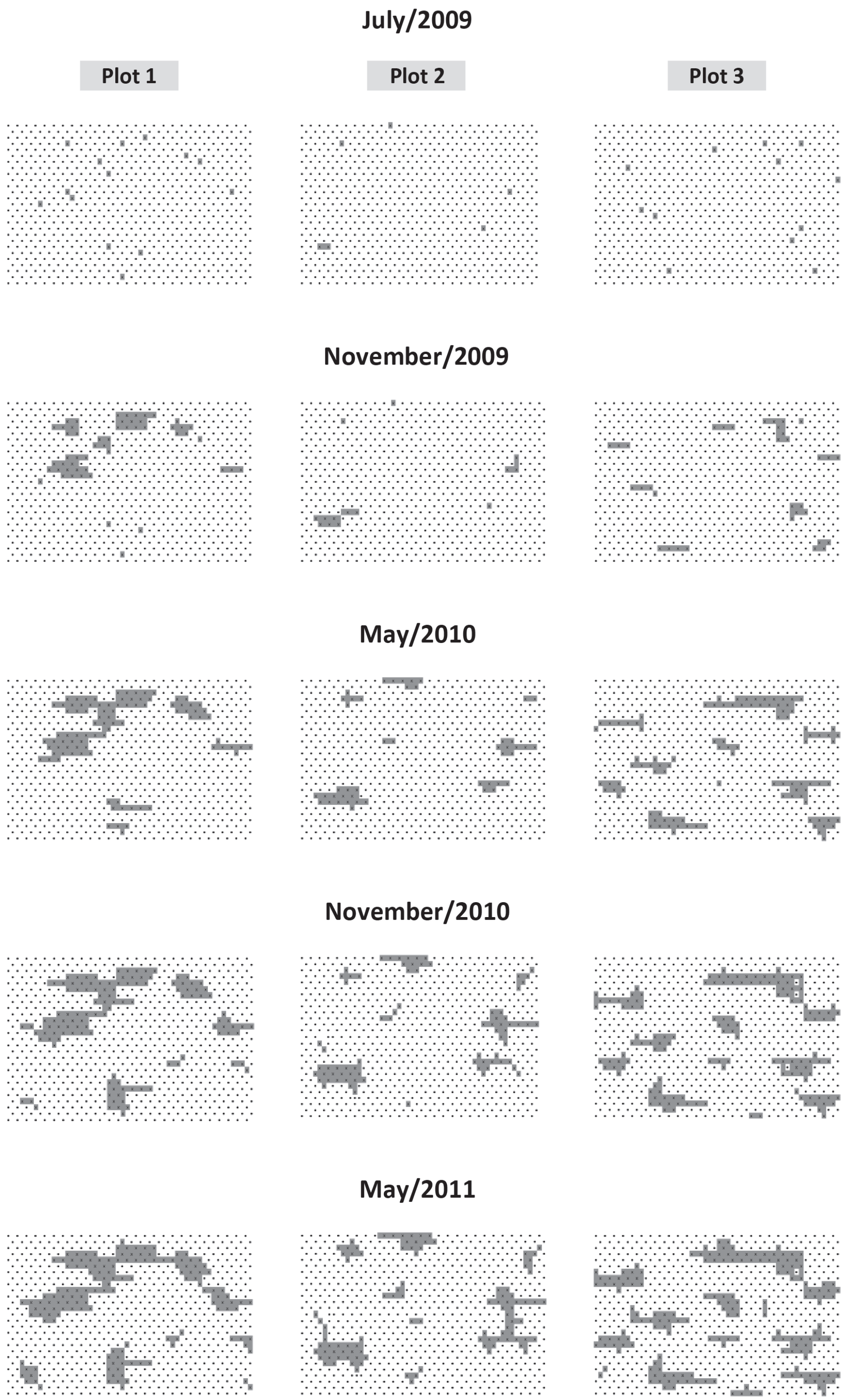

Figure 3 - Outbreaks of coconut stem bleeding detected by disease symptoms in the area of Neopolis, Sergipe, Brazil.

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