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Spatial distribution of cases of fatal yellowing on organic oil palm plantation

Distribuição espacial de casos de amarelecimento fatal em plantio orgânico de palma de óleo

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Abstract

This work studied the spatial dispersion of cases of fatal yellowing (FY) in an oil palm plantation in Acará, Pará State, Brazil. Data were collected monthly from two areas, divided in 16 quadrants each, for a 24 month period (2012-2013). In each quadrant, 138 plants were evaluated. The number of diseased plants was counted in twenty planting lines, and the spatial pattern of the disease was analyzed. The following spatial analysis techniques were used: common sequences of "*runs*", "*doublets*" and quadrants. We also determined the levels of disease aggregation in the sectors. Plants with FY had a spatial distribution aggregated in a lower line number when using the "run" analysis than when using the "*doublets*" method. Aggregation of FY cases was observed in 30% of the evaluated lines. The quadrant analysis confirmed the existence of case aggregation, with vertical and horizontal distribution. The existence of disease forming foci suggests that FY probably has biotic origin. Additional studies are required to confirm this hypothesis.
Key words: Dispersion. *Elaeis guineensis.* Epidemiology. Plant disease.

Resumo

O trabalho teve por objetivo estudar a dispersão espacial de casos de amarelecimento fatal (FY) em plantio de palma de óleo no município de Acará, Estado do Pará, Brasil. Os dados da incidência de FY foram coletados em inspeções mensais, durante vinte e quatro meses (2012 e 2013) em duas subáreas, com dezesseis quadrantes cada. Foram avaliadas 138 plantas em cada quadrante. Em vinte linhas de plantio foram contados o número de plantas doentes e feita análise do arranjo espacial da doença. As técnicas de análise espacial utilizadas foram: a sequências ordinárias de "*runs", "doublets"* e quadrantes. Também foram determinados índices de agregação da doença nas subáreas. Plantas com FY apresentaram distribuição espacial agregada em número menor de linha quando utilizando a metodologia de analise de "*runs*" que quando utilizada a metodologia de "*doublets*". Verificou-se que em 30% das linhas avaliadas houve agregação de casos de FY. A análise de quadrantes evidenciou a existência de agregação de casos, com distribuição vertical e horizontal. A ocorrência da doença formando reboleiras sugere que o FY tem origem biótica, sendo necessários mais estudos para confirmar a hipótese.
Palavras-chave: Dispersão. Doença de plantas. *Elaeis guineenses.* Epidemiologia.

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The African oil palm, *Elaeis guineensis* (Jacq.) is an oleaginous species with a high potential for oil production and great social and economic significance in several countries. This species has a lifespan of at least 25 years and greater oil productivity than other cultures (palm and palm kernel oil). Of all the existing oleaginous species that are cultivated, oil palm holds the first place for worldwide production above 56 million annual tons (MONTEIRO; HOMMA, 2014), with Indonesia as the largest producer, at 35.5 million tons per year (USDA, 2015). Brazil still has a low presence in the world palm oil market. Nevertheless, with more than 28.9 million hectares suitable for palm planting (mostly in its northern region) (ALVES et al., 2013), Brazil has space to significantly expand the oil palm planting area.

However, phytosanitary problems present an important risk to the expansion of this crop. The production system favors insect plagues and diseases, and fatal yellowing (FY), also known as bud rot (SALES, 2011) is a highly lethal disease for *E. guineenses*. Plants affected by FY start by developing a light yellowing in the basal leaflets of intermediary leaves (3, 4, 5 e 6). At later stages, there is necrosis in the leaflets' extremities, which evolves to complete leaf desiccation. Incidence of this disease is highest in the equatorial Amazon (Brazil, Colombia and Surinam). FY is responsible for decimating vast planting areas in several countries, and jeopardizes the development of new plantations in the Amazon (BOARI, 2008).

Fatal yellowing does not have a known etiologic agent. Studies attributing the cause of the disease to biotic factors (although with more than four decades of research) are inconclusive. Disease expression and development of symptoms may follow different stages, depending on the spatial and temporal environmental conditions of planting (DRENTH et al., 2013). Because each illness possesses its own characteristics, analyzing the spatial distribution of a disease may help to understand its origin and dissemination. Recent studies in Colombia described two stages of disease dispersion: in the first stage, random individuals are infected throughout the plantation; in the second stage, there is focal propagation of the disease from the palms infected in the previous stage. Thus, the pattern of distribution of FY in planting areas may contribute to the identification of its etiologic agent. This work aimed to study the arrangement and spatial distribution of FY in areas of commercial planting of *Elaeis guineensis* in Acará, Pará State, Brazil.

Research information was collected between 2012 and 2013, from two sectors of a plot in a farm belonging to Companhia Palmares da Amazônia (CPA), Acará, Pará State, Brazil, chosen due to the existence of FY cases at initial stages. The property is at an elevation of 18m, in coordinates 02° 16' 27" S and 48° 37' 29" W. Local climate is Köppen type Ami, with heavier rainfall from January to May and lighter rainfall from August to November (annual average 2,430 mm). Average annual temperature is approximately 26 ºC and average relative air humidity is 80%.

Oil palm planting followed a format compatible with organic production (FONSECA et al., 2009). Great genetic diversity was planted in the several plots. Plants were spaced 9 m in an equilateral triangle configuration. The selected plot contained homogeneous planting performed in 1996. Data on disease incidence was collected from two sectors (A and B) during 24 months (2012 and 2013) by searching for typical FY symptoms in each plant and counting plants with FY symptoms, along 20 planting lines. Presence or absence of symptoms and the relative position of each plant were recorded and used to build a disease map for each sector. The analysis included each case in a cumulative manner, meaning a plant marked as diseased remained as such in posterior assessments. The total number of diseased plants per line was used to define the pattern of FY dispersion in the plot.

The distribution pattern of diseased plants was assessed with the "*Ordinary runs*" and "*doublets*"

tests, according to Ferreira et al. (2009) and Carvalho (2013). The "*ordinary runs*" analysis evaluated the aggregation between immediately adjacent (within the lines) diseased plants. A "run" (*R*) is described as the sequence of one or more diseased plants. The number of expected "runs" *E(R)* for the null hypothesis of randomness is given by $E(U)=1 + [2m (N-m)/N]$, where *m* is the number of plants with FY and *N* is total number of plants per line. Standard deviation of *R*, for the null hypothesis, is given by $S(U)=\{2m(N- m)[2m(N-m)–N]/[N^2(N-1)]\}^{1/2}$. A normal standard test *Z*, where $Z(U)=[U-E(U)]/S(U)]$, was used to determine the significance of aggregation of diseased plants. $Z(U)<1.64$ ($P=0.05$) rejects the null hypothesis (random arrangement), favoring the alternative hypothesis (aggregated arrangement). Where:

U: run number; m: number of diseased plants; N: number of plants per line; *S*(U): standard deviation.

For the "*doublets*" test, a "doublet" were two adjacent diseased plants; three adjacent diseased plants are two "*doublets*", and so forth. The number of lines in each block, number of diseased plants and total number of plants were similar to the "*ordinary runs*" test. In this case, the expected number of "*doublets*" is given by: $E(D)=m(m-1)/N$, where:

D is the number of "*doublets*", *m* is the number of plants with FY symptoms and *N* is the total number of plants. Standard deviation of *D* is given by: $S(D)=\{[m(m-1)[N(N-1)+(2N(m-2)+N(m-2)(m-3)-(N-1)m(m-1)]/N^2(N-1)]\}^{1/2}$. The standardized value of $Z(D)=[(D+0,5-E(D)]/S(D)$ was calculated based on a normal distribution. $ZD>1.64$ (P=0.05) indicates an aggregated pattern and $ZD<1.64$ (P=0.05) indicates a random pattern, where: *D*: *doublet* number; m: number of diseased plants; N: number of plants in the line; *S*(*D*): standard deviation.

A sixteen-quadrant grid was defined for each sector (each quadrant had 138 plants) based on the spatial distribution map of the disease. A dispersion index (D), representing the relationship between (Variance (s^2)/Average (x)), was calculated. The spatial pattern is regular for (D<1), random for (D=1) and aggregated for (D>1).

The Morisita Index was used to establish the spatial distribution pattern of plants with FY. An index lower than 1.0 indicates a uniform distribution, an index of 1.0 indicates a random distribution and an index greater than 1.0 indicates an aggregated distribution. This index was chosen as it performs a better categorization of the dispersion of diseased plants in the area and because the size of the sampling area does not influence the spatial distribution of FY. The Morisita Index was calculated by the formula:

$$Id = n *[\Sigma x^2 – N]/[N * (N – 1)]$$

where: Id: Morisita Index; n: total number of sampled plots; N: total number of plants evaluated in n plots; x^2: squared number of individuals per plot. The Chi-square test was used to assess the significance of the Morisita Index (P=0.05), according to the formula:

$$\chi^2 = n *\Sigma x^2 (N– N)$$

where: χ^2: chi-square value; N, x^2 and n: previously defined. The following criterion was used to interpret the chi-square value: if the calculated value is lower than the tabled value, the Id is not significantly different from 1 and the disease will have a random distribution pattern. However, if the chi-square value is greater than the tabled value, the disease will tend to have an aggregated distribution pattern, if Id >1, or an uniform pattern, if Id<1.

The calculations for foci structure analysis (AFDS) were performed based on the spatial distribution map of plants with FY. Plants with symptoms immediately adjacent in a vertical, horizontal or diagonal proximity pattern were considered foci. We evaluated the number of unitary foci (those composed by one affected plant) (NUF); the number of foci in the area (NF); the number of plants per foci (NPF); the foci shape index (FSI); the foci aggregation index (FAI). The maximum number of occupied lines (Lf) and columns (Lc) was calculated for each designated foci, using FSI=[(Lf/

Lc)] and FAI=[(Lf*Lc)/NPF]. All analyses were performed in Microsoft Office Excel® 2010.

The first plants displaying FY symptoms were identified in April 2012. From the 11 cases initially identified, five occurred close to the access point; the rest occurred in the center of the plot, in a dispersed manner. FY studies performed by Laranjeira et al. (1998) and Van de Lande (1993) reported similar results. The biggest incidence of FY was registered in October, with 58% and 41% of cases, in 2012 and 2013, respectively.

Mapping the FY incidence enabled us to grasp the current situation in the plots studied. In sectors A and B, there were indices of aggregation of diseased plants.

Both the "*runs*" and "*doublets*" tests indicated that the diseased plants are randomly distributed in the beginning (in the twenty lines), and that aggregation occurs later on. There was significant case aggregation in six lines according to the "*ordinary runs*" test (P = 0.05) and in nine lines, according to the "*doublets*" test. According to Bergamin Filho et al. (2007), a low number of "*runs*" tends to define aggregated arrangements.

There was more aggregation in the *"doublets"* analysis than in the *"runs"* study. An aggregated spatial arrangement (Z(D)> 1.64; P=0.05) was found in the final evaluation for seven lines. Two diseased adjacent plants are necessary to identify a *"doublet"*.

The dispersion index (D) for the evaluated sectors (138 plants grouped in sixteen quadrants each) was 6.19 (average 6.44; variance 39.86) for sector A and 5.4 (average 8.25; variance 44.6) for sector B. The Morisita Index was significantly (chi-square) higher than 1 (1.76 for sector A and 1.5 for sector B), indicating an aggregated spatial pattern. The dispersion index (D) satisfied the spatial pattern description for the disease, as aggregation is observed in the distribution map.

We focused on plants that displayed FY symptoms, immediately adjacent in a vertical, horizontal or diagonal proximity pattern. Other researchers have adopted these same criteria for different pathosystems (JESUS JUNIOR; BASSANEZI, 2004; FERREIRA et al., 2009).

In each sector, 2,208 plants were mapped, and the number of diseased plants was 104 for sector A and 134 for sector B. In the beginning, unitary foci or foci with two plants prevailed in the planting lines. Later on, there was aggregation of up to five plants per line. The existence of unitary foci decreased with disease progression (a total of 69 foci were randomly distributed in the two sectors).

The arrangement of the disease in small foci may indicate that there was insufficient time for diseased plants to become sources of greater subsequent infections. Tumura et al. (2012) obtained FSI<1 for *Ceratocystis* withering in the eucalyptus, and observed a greater distribution in the planting line (different from our results).

A greater incidence of unitary foci at the beginning of the disease was also observed for epidemics of *Ceratocystis* em withering in eucalyptus (TUMURA et al., 2012). For plots of citrus with up to 2% of plants with sudden death symptoms, 85% of the foci occur in single plants (JESUS JUNIOR; BASSANEZI, 2004).

A maximum of eight unitary foci per line was observed, the average varying between 2.88 and 4.88. This is similar to what Jesus Junior and Bassanezi (2004) reported for sudden death in citrus plots, that most foci include more than one plant. These authors found that as the number of cases increases, the number of foci decreases. Van de Lande (1993) also reported significant FY aggregation in Victoria, Suriname, for a 0.7% incidence (P<0.05). Incidences of 1.5%, 8.3% and 9.3% in other blocks were also sufficient for detection of significant aggregation.

Table 1 shows the results for the analysis of foci dynamics and structure for sector A. FAI is close to 1 in most quadrants, indicating the existence of aggregated foci and reinforcing the results obtained with the aggregation indexes.

Table 1. Analysis of foci dynamics and structure (AFDS) for *Elaeis guineensis* plants with fatal yellowing, in sector A: quadrant number; number of diseased plants (NDP); percentage of diseased plants (%DP); number of unitary foci (NUF); number of foci (NF); number of plants per foci (NPF); maximum number of diseased plants in columns (Lc); maximum number of diseased plants in lines (Lf); foci shape index (FSI); foci aggregation index (FAI).

Quadrant	NDP	%DP	NUF	NF	NPF	Lc	Lf	FSI	FAI
1	15	0.11	5	7	5	2	4	2.00	1.60
2	9	0.07	0	4	4	2	2	1.00	1.00
3	23	0.17	0	6	7	2	3	1.50	0.86
4	14	0.10	1	6	3	2	3	1.50	2.00
5	9	0.07	2	5	3	2	2	1.00	1.33
6	6	0.04	4	5	2	2	1	0.50	1.00
7	5	0.04	1	2	2	1	2	2.00	1.00
8	8	0.06	0	2	5	2	3	1.50	1.20
9	7	0.05	0	1	7	2	3	1.50	0.86
10	0	0.00	0	0	0	0	0	0.00	0.00
11	3	0.02	3	3	1	1	1	1.00	1.00
12	1	0.01	0	1	1	1	1	1.00	1.00
13	3	0.02	3	3	1	1	1	1.00	1.00
14	1	0.01	1	1	1	1	1	1.00	1.00
15	0	0.00	0	0	0	0	0	0.00	0.00
16	0	0.00	0	0	0	0	0	0.00	0.00

The area maps indicate a radial progression for foci growth. The greatest number of cases occur in the lines. Carvalho et al. (2012) obtained similar results when studying the distribution pattern for coconut stem-bleeding cases, reporting case aggregation with compact foci (FAI close to 1).

The results of the analysis of foci dynamics and structure for sector B (Table 2) are similar to those obtained for sector A, reinforcing the aggregation indexes for the disease in the planting area.

An inverse relationship between NUF and NPF, associated to increased incidence, indicates transmission between proximal plants after the disease is established. This is characteristic of diseases with a biotic origin, where diseased plants influence the condition of immediately adjacent plants (CYSNE, 2009). Jesus Junior and Bassanezi (2004) studied sudden death in citrus, reporting results similar to those found in this study.

We conclude that there was aggregation of FY cases in 30% of the evaluated lines; plants with FY had a spatial distribution aggregated in a lower line number when using the "*runs*" analysis than when using the "*doublets"* method; the quadrant analysis confirmed the existence of case aggregation, with vertical and horizontal distribution. The existence of disease forming foci suggests that FY probably has biotic origin. While additional studies are required to confirm this hypothesis, the conditions of the roots in plants with FY throughout the year may help clarifying the origin of the disease.

Table 2. Analysis of foci dynamics and structure (AFDS) for *Elaeis guineensis* plants with fatal yellowing, sector B: quadrant number; number of diseased plants (NDP); percentage of diseased plants (%DP); number of unitary foci (NUF); number of foci (NF); number of plants per foci (NPF); maximum number of diseased plants in columns (Lc); maximum number of diseased plants in lines (Lf); foci shape index (FSI); foci aggregation index (FAI).

Quadrant	NDP	%DP	NUF	NF	NPF	Lc	Lf	FSI	FAI
1	5	0.04	3	4	2	1	2	2.00	1.00
2	1	0.01	1	1	1	1	1	1.00	1.00
3	1	0.01	1	1	1	1	1	1.00	1.00
4	3	0.02	1	2	2	1	2	2.00	1.00
5	24	0.17	4	10	9	2	4	2.00	0.89
6	6	0.04	4	5	2	1	2	2.00	1.00
7	7	0.05	3	6	2	1	2	2.00	1.00
8	7	0.05	4	5	2	1	1	1.00	0.50
9	19	0.14	3	7	5	2	3	1.50	1.20
10	11	0.08	2	5	5	2	4	2.00	1.60
11	13	0.09	4	8	3	2	1	0.50	0.67
12	4	0.03	2	3	2	1	1	1.00	0.50
13	19	0.14	8	10	3	2	3	1.50	2.00
14	8	0.06	5	6	3	1	2	2.00	0.67
15	7	0.05	4	5	2	2	1	0.50	1.00
16	0	0.00	0	0	0	0	0	0.00	0.00

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