

Spatial Analysis and Population Dynamics of *Haplaxius crudus* (Hemiptera: Cixiidae) in Coconut Amazon

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Abstract

Haplaxius crudus is the primary vector of the phytoplasma that causes the Lethal Yellowing of Coconut, a disease that has become a barrier to the establishment of large coconut plantations in the world. The objective of this study was to determine the spatial distribution pattern and population dynamics of *H. crudus* adults by correlating them with the abiotic factors, such as temperature and rainfall in commercial dwarf coconut palm plantations. Collection of American palm cixiid was carried out using yellow sticky traps fixed to the abaxial part of the coconut leaves. The sampled plants were georeferenced to obtain the geographic coordinates and geostatistical analysis, besides the planialtimetric survey of the experimental plot for the preparation of the map that shows the topographic conformation of the terrain. Insects were found at the study site throughout the year, showing greater abundance in months when the average monthly temperatures and rainfall were not significant. The climatic factors showed a correlation with the total of insects, positive for the temperature, with 0.733 and negative for the precipitation with -0.606. The spatial distribution of *H. crudus* displayed an aggregate pattern, explained by the spherical model. The infestation focus occurred in the periphery of the plot, near a *Brachiaria humidicola* pasture. It was concluded that the infestation of the *H. crudus* showed a behavior directly related to the local climatic conditions and the aggregate spatial distribution explained by the spherical model, therefore, forming shrubs with a radius of 154 to 190 m, with areas of influence between 7.45 and 17.80 ha, which coincide with the lower part of the terrain.

Keywords: altimetry, American palm cixiid, geostatistics, pest monitoring, kriging

1. Introduction

The coconut (*Cocos nucifera* L.) is a monocotyledon plant belonging to the Arecaceae family. This plant exhibits arboreal behavior, with a stem-like trunk. It is originated of the islands in Southeast Asia. Its cultivation is considered an agricultural activity of great importance, mainly in countries in economic development (Harries & Clement, 2013). The state of Pará is the largest coconut producer in the Brazilian North region and the fourth largest in Brazil, with a planted area of 18,595 ha and production of 173,788 tons of fruits (IBGE, 2018; FAOSTAT, 2016).

A sharp drop was found in the worldwide coconut productivity caused by the rapid spread of a disease termed Lethal Yellowing. The primary obstacles for controlling such disease are its rapid progress and high lethality, besides the lack of studies on the transmission (Ramos-Hernández et al., 2018). The first symptoms of the disease are the abortion of the fruits, followed by the blackened color, resulting in the reduction of seed viability. The visible symptoms are the necrosis of the inflorescences and the so-called yellowing or bronzing of the leaves.

These leaves undergo dehydration processes, progressing towards their falling (Bertaccini et al., 2014). Death in coconut palms and other palm trees may occur approximately four months after the onset of the first symptoms (Meyerdirk & Hart, 1982; Gurr et al., 2016). The disease is caused by a phytopathogen that present cell composition similar to the bacteria, belonging to the group of phytoplasmas, whose translocation within the plant is through the phloem, and the transmission from one plant to another occurs by the activity of a vector (Waters & Hunt, 1980; Abeysinghe et al., 2016), which is the *Haplaxius crudus* Van Duzee 1907 (Hemiptera: Auchenorrhyncha: Cixiidae). The disease is spread by *H. crudus*, considered the main vector insect. This fact does not exclude the possibility of transmission through other sap-sucking insects, like other species of American palm cixiid, which is caused by the habit of feeding on the floemamatic sap, a local where its causative phytoplasma is found (Wheeler & Wilson, 2014; Narváez et al., 2018).

According to Tsai and Kirsch (1978), Howard and Wilson (2001), and Howard (2012), the nymphs of this hemipterus feed on the sap elaborated in the roots of some grass species and some Cyperaceae, with an average development time of approximately 80 days at 24 °C and 52 days at 30 °C under laboratory conditions in experiments of studies conducted in Florida. In the adult stage, it can be found in the abaxial part of the palm leaves (Gitau et al., 2009). They feed on the lower parts of the leaves, where they also find shelter, or in partially hidden parts of the host plants close to the soil (Kramer, 1979; Perilla-Henao & Casteel, 2016). Several control methods can effectively carry out the populational reduction or even the extinction of the vector, *H. crudus*. These include especially chemical and cultural controls, with the removal or replacement of the forage species occurring at the site of infestation and surroundings, aiming at restricting the reproductive activity and initial development of the pest (Pardey & Arango, 2016).

In such cases, geostatistics becomes an excellent tool for the search of patterns in the form of distribution of many insect pests in different cultures or even in aid of the prevention of possible infestations, where the most proper form of management can be carried out using the results achieved in the study. Several works in this line of study such as those of Fernandes et al. (2003), Dinardo-Miranda et al. (2007), Trindade et al. (2017), and Farias et al. (2018) have been carried out based on the use of this tool as an essential aid to integrated pest management. For the implementation of sustainable management and to reduce costs with the use of pesticides, it is paramount to master the knowledge regarding the population dynamics and spatial and temporal distribution of insect-pests in commercial areas of large crops. This information provides greater precision to the adopted phytosanitary control strategy, controlling the direct problem in the infestation focus (Dal Prá et al., 2011). Therefore, geostatistical analysis through incidence maps and distribution of insects that cause damage to monoculture sites provides a great help to control methods, granting them greater efficacy (Duarte et al., 2015). The possibility of the dissemination of Lethal Yellowing throughout South America is a reality that concerns large coconut farmers in Brazil. Hence, there is a need to use new phytosanitary monitoring technologies to prevent this disease. Therefore, the objective of this study was to study the spatial distribution and the annual population fluctuation of *H. crudus* in commercial dwarf coconut plantation area, using geostatistics as an analysis tool.

2. Material and Methods

2.1 Experimental Area

The study was carried out in commercial areas planted with Brazilian dwarf coconut located at 01°13'40.16" S and 48°02'54.35" W, (Figure 1) using spacing of 7.5 m. The region is characterized by high rainfall rates, with values up to 3,000 mm and average relative humidity of approximately 80%. The climatic classification according to Köppen-Geiger is of the Af type (Sema, 2017), in which the rainy season occurs from January to May and the dry season is from June to December. It also displays secondary vegetation of the *Capoeira* type with foci of pastures. The most predominant soil is the quartzarenic Neosol, with adequate drainage and high weathering levels (Embrapa, 2006).

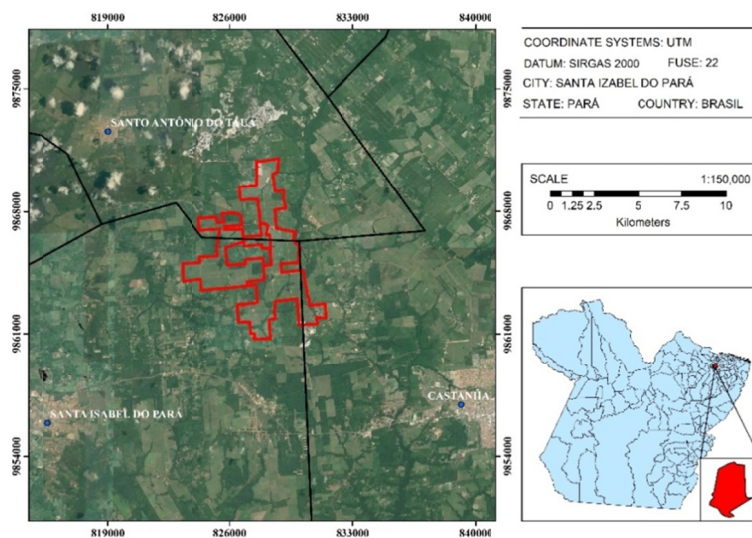


Figure 1. Study area map - Eastern Amazon, Brazil

2.2 Experimental Design

In order to select the study area, a preliminary survey of the aerial entomofauna was carried out in 8 plots with different environments, where the results showed a high incidence of *H. crudus* in the vicinity of a water body, which comprised 3 out of the 8 plots, among them one was chosen at random.

The selected area had 19.21 ha with 6-year-old plants, near a dam and areas with *Brachiaria humidicola* (Rendle) Schweik. The meteorological parameters, such as rainfall (mm) and temperature ($^{\circ}\text{C}$), were obtained from the meteorological station owned by Sococo Agroindústrias da Amazônia.

Forty yellow sticky traps in the 23×28 cm (Mark ISCA) were installed. They were set at the base of the canopies of the coconut palms at 1.50 m from the ground and arranged in 60×78 m so that we could obtain the best possible representativeness, as described by Roberto et al. (1997). The taxonomic identification was performed using a stereoscopic microscope and dichotomous keys described by Triplehorn and Johnson (2015) and Rafael et al. (2012). For confirmation of the species, some specimens were selected and sent to the Research Center, Embrapa Tabuleiros Costeiros, a pioneer of finding the vector insect in Brazil (Silva et al., 2019).

Traps were installed every fortnight over a year (October 2017 to September 2018). After collection, the traps were conditioned in transparent plastic bags and stored in a cold room at 5°C , for later quantification and taxonomy.

2.3 Georeferencing and Planialtimetric Survey of the Study Area

Each plant that received a trap georeferenced within the Geographic Information System (GIS), which is based on the Latitude and Longitude crossing. Through this information, one can generate specific geographical positions. The points generated by the coordinates were transformed into UTM (Universal Transverse of Mercator) according to the rectangular system. In the geo-referencing, the Garmin GPS navigation device, model Montana 680 was used (Farias et al., 2002)

A survey of the altimetry of the experimental plot was carried out using the Trimble Geostatic GPS apparatus, model R6, with a $3 \text{ mm} + 0.1 \text{ ppm RMS}$ precision. For the execution of the areascanning, 30 points were collected, out of which 1 point was from each of the vertices of the plot was tracked. Regarding the preparation of the digital map with more precise information, the data obtained were processed in the Platform IBGE-PPP (Precision Point Processing). The values of the coordinates, in Northing, Easting and Altitude UTM of each sampling point were obtained so that they all belonged to the spindle 22. The minimum curvature interpolator, which is a digitized terrain construction algorithm, was used to the construction of the digitized map of the plot and for quotas obtaining (Press et al., 1988).

The scanned map of each point of the traps was carried out using SURFER 14.0 software (Brandão, 2018) (Figure 2).

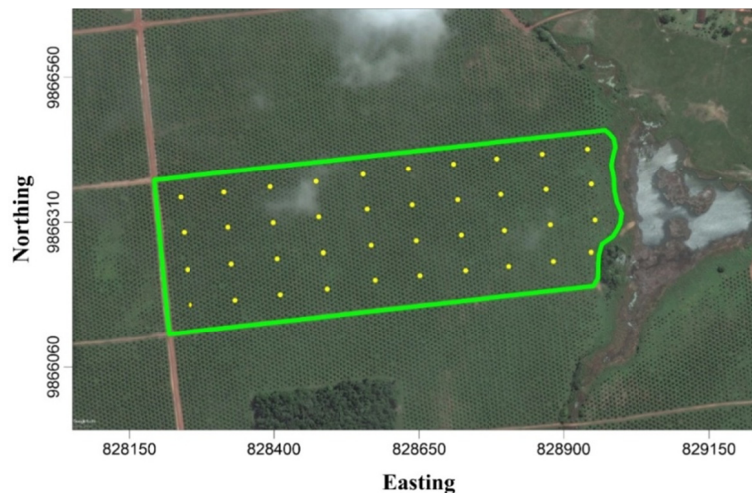


Figure 2. Arrangement of the adhesive traps for the capture of *H. crudus* in a commercial coconut plantation area, Eastern Amazon, Brazil

2.4 Geostatic Analysis

The semivariograms were adjusted for each of the twelve evaluation months, and then the Kriging maps were made (Vieira et al., 1983). The regionalized variable Z considered in the study was the number of *H. crudus* adults caught in the adhesive traps, which varied according to the geographical positions determined by the variables longitude (X) and latitude (Y). The spatial dependence between neighboring pairs of points was calculated through the semivariance, estimated by Equation 1:

$$\gamma^*(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(X_i) - Z(X_i + h)]^2 \quad (1)$$

Where, $\gamma(h)$ is the semivariance calculated by the distance h ; $N(h)$ is the total number of traps analyzed, which are separated by a distance h . In the case of spatially dependent variables, the values $[Z(x_i) - Z(x_i + h)]^2$, increase until reaching the point of stability (Farias et al., 2002b).

The parameters established for the analysis of the semivariograms were the plateau ($C_0 + C_1$), the nugget effect (C_0) and the range (a). The plateau is the value of the semivariogram corresponding to its range. The nugget effect is the semivariance between adjacent pairs of points and the range measures the maximum distance that may exist from the spatial dependence. The spatial dependence index (SDI) k ($C_0/(C_0+C_1)$ ratio) of each evaluation was also calculated in this study. It is classified as weak spatial dependence $SDI > 75\%$, moderate $25\% \leq SDI \leq 75\%$ and strong $SDI < 25\%$ (Cambardella et al., 1994; Landim, 1998; Farias et al., 2004; Pinho et al., 2016).

For each month of sampling, the experimental semivariograms were made, and the best mathematical models were evaluated, taking into account the refining of the adjustment and its value of Determination Coefficient in which Linear, Exponential, Gaussian and Spherical models stood out (Journel & Huijbregts, 2003). After obtaining the appropriate models, it is possible to make maps by using the ordinary Kriging method, to observe the incidence and the spatial arrangement of the insect in the experimental plot, through the interpolation methodology (Vieira et al., 1983). For the calculation, the ordinary kriging estimator was used, Equation 2:

$$Z^*(x_0) = \sum_{i=1}^N \lambda_i Z(x_i) \quad (2)$$

Where, Z^* was the estimate for the location, and x_0 was the linearly combined values of the neighboring measures. N symbolized the number of measured values related to the estimate, λ_i was the moderation associated with the measured values. In this method, the weights were calculated under two restriction conditionals: where the variance of what is estimated is as minimum as possible and that the estimator does not present a tendency or inclination (Journel & Huijbregts, 2003).

The most adequate model to the parameters was selected through standardization and adjustment of the data, shown in Equation 3:

$$\gamma(h) = \{C_0 + C_1 \cdot \text{Modelo}(\alpha)\} \quad (3)$$

Where, C_0 was the minimum semivariance, $(C_0 + C_1)$ was the maximum semivariance, α was the aggregation range, and h was the distance that separates the pairs of points (Yamamoto & Landim, 2013).

The significance of the Pearson's correlations of the population means of the insects and the average annual and monthly rainfalls was performed using the Student's T-test at $p > 0.05$. EXCEL 2013 and STATISTICA 8.0 were the software used in the study.

3. Results

A total of 850 *H. crudus* specimens were collected over the complete collection period. A reduction was observed in the number of insects according to the rise in the rainfall and temperature (Figure 3). In December 2017 and September 2018, the highest population peaks of the insect were recorded, totaling 170 and 212 respectively. The lowest quantities were obtained in February and May 2018, with only 2 and 3 specimens.

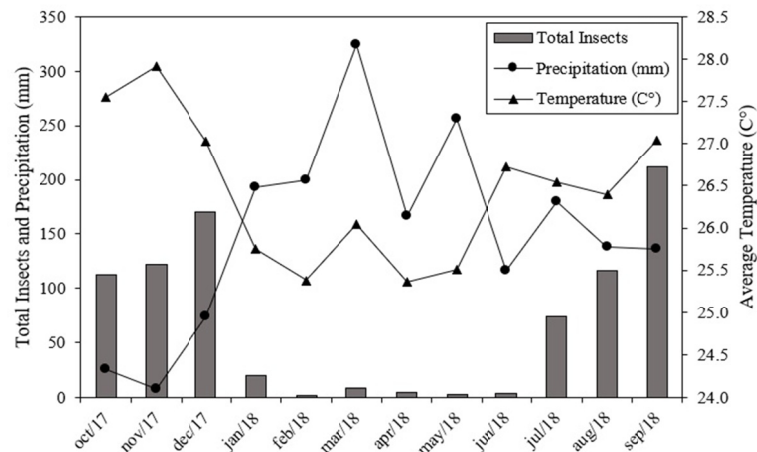


Figure 3. Monthly population fluctuation of *H. crudus* collected in green dwarf coconut plantation in Brazil, rainfall, and temperature. Eastern Amazon, Brazil

October, November and December 2017 and June, August and September 2018 showed the lowest average rainfall of 72.6 mm. November displayed the most critical rainfall in the evaluated period, only 8 mm. In January, February, March, April, May, and July, the average was 220 mm, directly affecting the occurrence of insects (Figure 3).

It was verified that the population dynamics of *H. crudus* had a strong relationship with the monthly meteorological variables, rainfall, and temperature. The population of *H. crudus* reduced as the amount of rainfall raised, and the temperature decreased. Pearson's correlation between the monthly average of insects and the accumulated rainfall value per month showed a value of -0.606 ($p \geq 0.05$), which indicated a negative correlation between the factors. The significant value of 0.733 ($p \geq 0.05$) indicated a positive correlation, which was obtained when the average monthly temperature variable was correlated with the monthly average of insects (Figures 4 and 5).

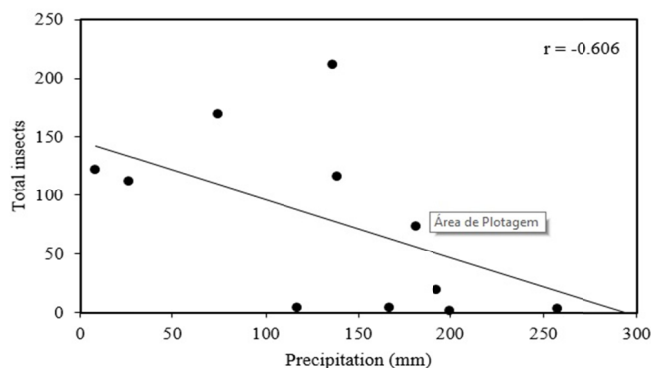


Figure 4. Correlation between the monthly total of *Haplaxius crudus* individuals collected in commercial green dwarf coconut areas of Brazil and the average monthly rainfall. Eastern Amazon, Brazil

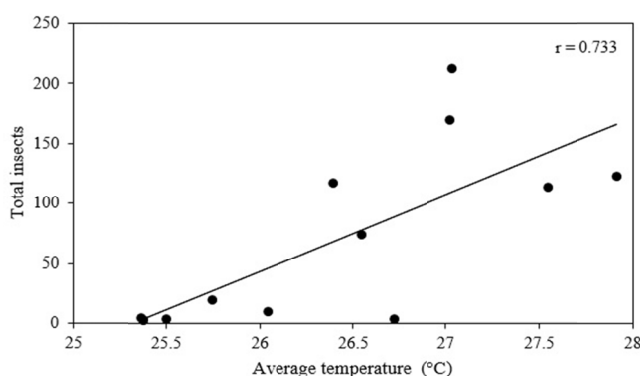


Figure 5. Correlation between the monthly total of *Haplaxius crudus* individuals collected in commercial green dwarf coconut areas of Brazil and the average monthly temperature. Eastern Amazon, Brazil

It was observed that the minimum range value was at 154 meters of distance with 7.45 ha of area. It was influenced by insect infestation, confirming that the network formed by the spatial distribution of the traps in the field was considered satisfactory, therefore, demonstrating that an aggregated spatial distribution. However, the maximum range was 238 meters with an area of influence of 17.80 ha (Table 1).

Table 1. Semivariogram parameters, range area, coefficient of determination (R^2), spatial dependence index and experimental model for geostatistics analysis. Eastern Amazon, Brazil

Evaluation month	Parameters			Reach Area (ha)	R^2	k	Model	Degree of Dependency
	C0	C1	a (m)					
Oct/17	8	12.7	175	9.62	0.97	0.386	Spherical	Moderate
Nov/17	0	94	160	8.04	0.95	0.000	Spherical	Weak
Dec/17	0	19.7	190	11.34	0.99	0.000	Spherical	Weak
Jan/18	Without adjustment							
Feb/18	Without adjustment							
Mar/18	Without adjustment							
Apr/18	Without adjustment							
May/18	Without adjustment							
Jun/18	Without adjustment							
Jul/18	0	14.2	238	17.80	0.99	0.000	Spherical	Weak
Aug/18	6.8	15.4	154	7.45	0.95	0.306	Spherical	Moderate
Sep/18	10	136	190	11.34	0.97	0.068	Spherical	Weak

Note. Calculated by $\pi \cdot r^2$, in which $\pi = 3.14159$ and $r = a$; Relationship between C0/(C1 + C0).

Among the mathematical models most applied to data referring to biotic parameters, the spherical was the one that offered the best fit to the experimental semivariograms. These showed a coefficient of determination (R^2) that oscillated between the values of 0.95 to 0.99. The parameters obtained by the spherical model in relation to the occurrence of the *H. crudus* insect presented all coefficients of determination (R^2) greater than 0.90 (Figure 6).

The adjustment was performed using the spherical model for each of the 12 months; however, the rainy season months, from January to June, did not allow model adjusting due to the small number of insects captured, that is, because these months did not show a significant number of specimens.

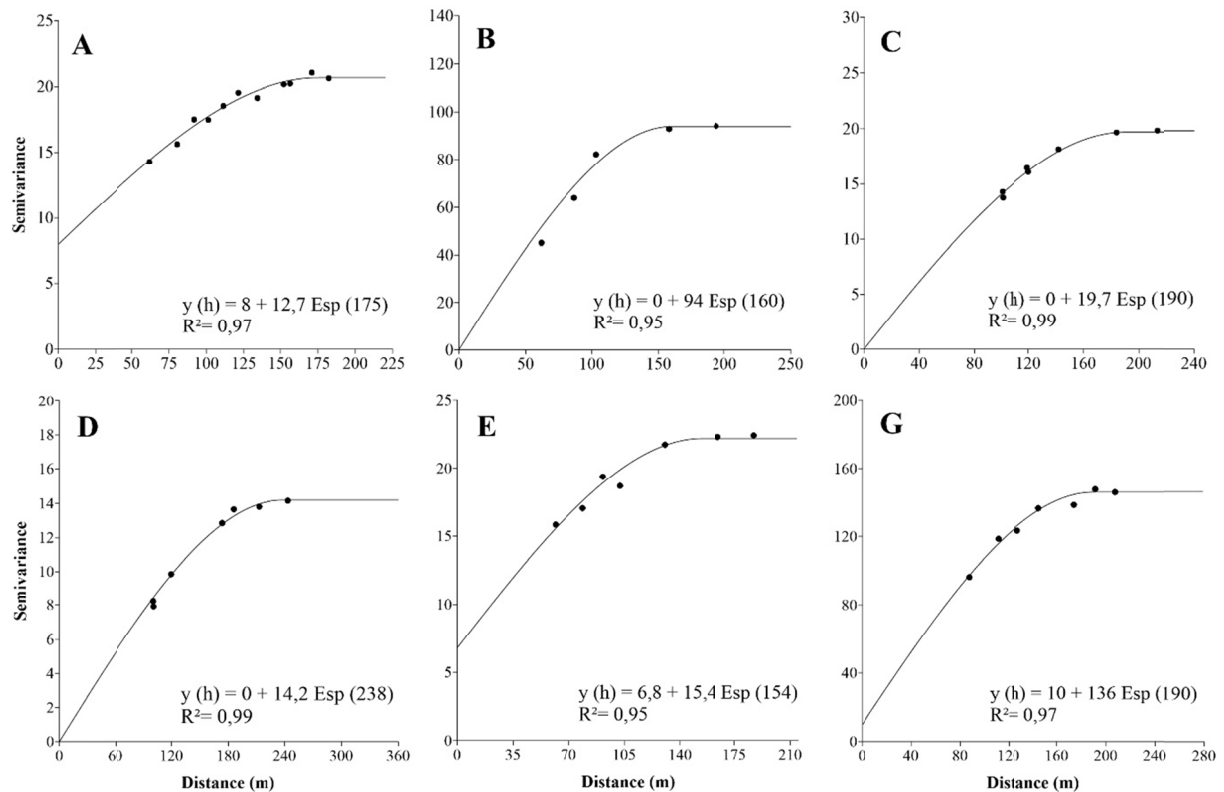


Figure 6. Semivariograms related to *Haploxius crudus* infestation in dwarf coconut commercial crops in Brazil, in relation to October (A), November (B), December (C) 2017 and July (D), August (E) and September (F) 2018. Eastern Amazon, Brazil

It was observed that from the surface maps, the infestation density and the positioning of the insects had undergone small variations over time and change of climatic conditions. However, they were usually found to be coincidentally concentrated between the two lower levels of the area, 12 and 13 m, respectively, where the area was characterized by humid soil and small foci of *B. humidicola* grass. In addition, the maps showed areas of aggregation of the pest, with the formation of a bush (Figures 7 and 8).

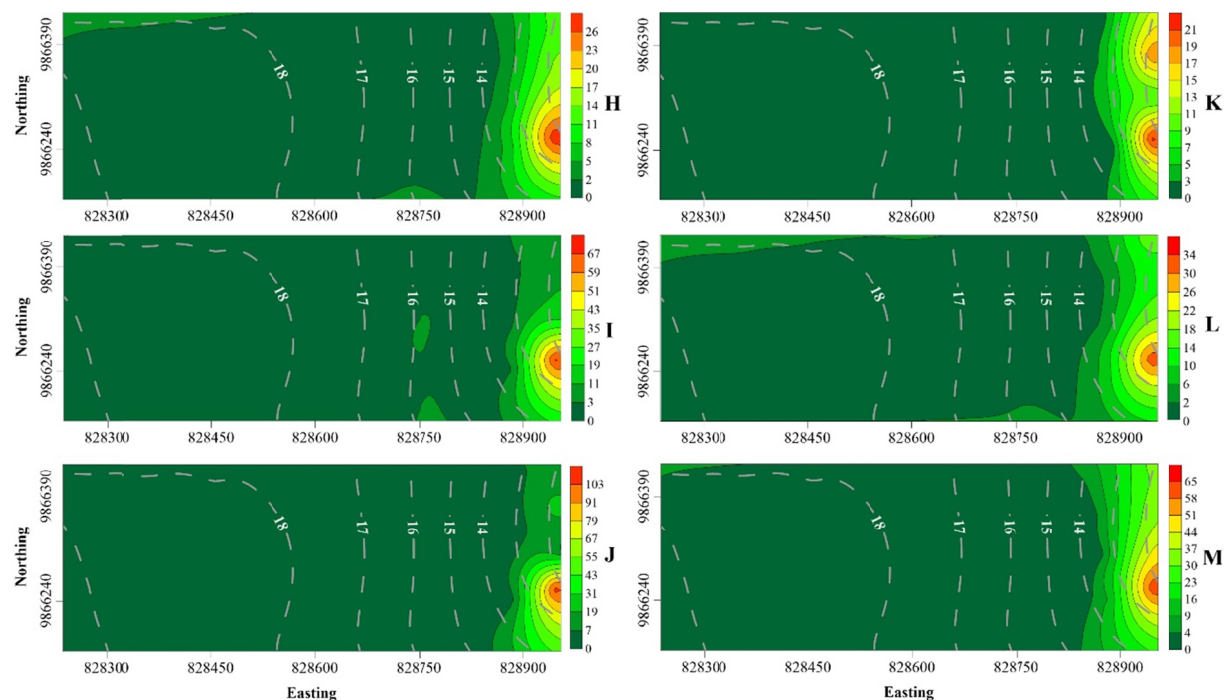


Figure 7. Kikring map of the spatial distribution of *Haplaxus crudus* and altimetry (dotted line) in a green dwarf coconut commercial crop in Brazil in October (H), November (I), December (J) 2017 and July (K), August (L) and September (M) 2018, Eastern Amazon, Brazil.

4. Discussion

The use of dispersion graphs (Figures 4 and 5), which shows a positive significant correlation between *H. crudus* population and the temperature and negative one for precipitation, the influence of biotic and abiotic factors has become evident. Factors already observed by Paradell et al. (2014), when reporting that American palm cixiid community suffers an increase in its population when the temperature, photoperiod, and air humidity factors are simultaneously raised. Although those variables are intrinsic, they are not the only ones that affect the population dynamics of these insects. Indeed, predation, interspecific competition, parasitism, among others seem to act in conjunction with meteorological factors, shaping the species distribution. The *H. crudus* population fluctuation is directly related to the climatic variations of the environment. The adults usually may be found all over the year in the abbatial portion of palm leaves, but they are more abundant in periods with lower rainfall (Moreno et al., 2014). Such a statement is shown in this study for *H. crudus*.

Climatological factors strongly influence the natural development of phytophagous insects, and these factors intrinsically interfere in the processes of oviposition, feeding, growth, and reproduction. In many cases, variables such as rainfall and temperature affect the population dynamics of insect pests and non-pests (Rahmathulla et al., 2012). Such fact confirms the behavior of *H. crudus* in this study, as it is a heterovoltin species, that is, the number of generations being affected by temperature, which is in agreement with what was reported by Halbert et al. (2014). Results similar to those obtained in this work were also shown by Silva et al. (2018), when they evaluated the seasonal variation of community of the suborder Auchenorrhyncha in Brazilian dwarf coconut trees, finding similarities to the present study, especially when the relative contribution of the abiotic factors in the explanation of the composition of these communities, in which *H. crudus* is inserted, because the authors demonstrated that insects of this suborder are more abundant immediately after the months that presented a high amount of rainfall and when the average monthly temperatures suffered a small raise.

Some studies have already shown this behavior for other sucking insects. Regarding the results obtained with the spatial distribution of *H. crudus*, an aggregate behavior was found. Generally, sucking insects of the suborder Auchenorrhyncha present this type of pattern. Some studies have already demonstrated this behavior for other sucking insects. It should be stressed the works of Oliveira et al. (2016), when studying the spatial behavior of *Empoasca kameri* adult insects (Ross & Moore, 1957) (Hemiptera: Cicadellidae) in physic nut and that of Leal et al. (2010), with plants showing greening disease (Huanglongbing/HLB) which is transmitted by *Diaphorina citri*

Kuwayama (Hemiptera: Psyllidae), and also obtained aggregate distribution. This model of distribution is the one that best suits the spatial behavior of these insects since this shows the foci of infestation in the form of concentric shrubs that tend to expand in all directions according to the population growth of the pest. By using the kriging maps of the spatial arrangement of *H. crudus* it was possible to observe a preference of the insect population on the periphery of the plot, closer to the pasture, where *B. humidicola* can be found. Some authors, such as Hernandez et al. (2018), studied the weeds used by *H. crudus* in a coconut pathosystem in southern Mexico and found that *B. humidicola* is one of the leading host species of the pest and suggested that the use of integrated management should be considered important when this grass is found in areas of coconut production. Also, after hatching of *H. crudus* nymphs, they descend to the soil surface and develop in the roots of the grass. Humid locals and grass with longer foliage are the most preferred locals (Howard, 2015), a fact confirmed in this study, as a greater aggregation of American palm cixiid were found in the lower and humid areas. Considering that the location of the point where the greatest infestation practically remains unchanged, it is suggested that this is the result of the dispersal behavior of this pest that occurs mainly through wind currents due to the low capacity of flight.

The altimetric survey for the analysis of the behavioral pattern of spatial distribution of the displayed results that demonstrate a clear preference for the areas of lower altitude, where there is a greater accumulation of moisture in the soil, which makes this environment conducive to the development of the insect. It should be noted that individuals of the Auchenorrhyncha suborder are necessarily sap-suckers that in their initial development depend on the environmental conditions where they are living in such as in the experiment by Latini et al. (2019), who demonstrated that the control of nymph-stage *H. crudus* should be carried out after hatching and before the first stages of larva, when they depend directly on the humidity. Therefore, reinforcing the need for constant phytosanitary monitoring in areas with similar characteristics.

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