SPATIAL DISTRIBUTION OF THE COFFEE-LEAF-MINER (*Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842)) IN AN ORGANIC COFFEE (*Coffea arabica* L.) FIELD IN FORMATION

João Domingos Scalon[1], Gabriella de Freitas Alves[2], Maria Betania Lopes Avelar[3], Mauricio Sergio Zacarias[4]

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ABSTRACT: Coffee production has been one of the economy pillars of many tropical countries. Unfortunately, this crop is susceptible to infestation by the coffee-leaf-miner **(***Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842)) which causes severe damage to coffee plantations with losses that may reach 80% of the total production. In recent years, researchers have been trying to develop practices for minimizing the use of pesticides in the coffee-leaf-miner control. It is well known that the understanding of the spatial distribution of insects may be important in the context of biological control of pests. The aim of this work is to use spatial statistical methods for characterizing the spatial distribution of the coffee-leaf-miner in a plantation of organic coffee **(***Coffea arabica* L.). This work uses the number of mined leaves taken from a grid of 35 sampling locations from one hectare of an organic plantation of coffee in the second year of its implantation during the annual peak population (September 2006) of the coffee-leaf-miner. A geostatistical method (semivariogram) was used to characterize the spatial variability of the coffee-leaf-miner in an organic coffee field in formation. The results showed that the coffee-leaf-miner population was randomly distributed in the field during the annual peak population.

Key words**:** Entomology, geostatistics, semivariogram, biological control.

1 INTRODUCTION

Coffee production is an economic mainstay for many countries in the world and with an annual estimated retail value of over US$ 70 billion it is surpassed only by oil. Brazil is the world's largest producer and exporter of coffee, being responsible for about 25 % of the world production. In Brazil there are an estimated six billion coffee trees, occupying an area of over 2.5 million hectares and, therefore, coffee production has been one of the pillars of the Brazilian economy for many years (LEWIN; GIOVANNUCCI; VARANGIS, 2004).

The genus *Coffea* consists of over 90 species but only two species, *Coffea arabica* L. and *Coffea canephora* Pierre (ex Froehner), also known as robusta, are commercially traded, with *C. arabica* comprising approximately 65% of coffee production (BRIDSON; VERCOURT, 1988). It is well known that both species are susceptible to fungal and insect pests. More than 850 insects have been reported to attack coffee (LE PELLEY, 1973). Among these pests, the most important throughout Brazil is the coffee-leaf-miner, *Leucoptera coffeella* (Guérin Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae). The coffee-leaf-miner causes severe damage to coffee crops. It is estimated that the loss in yield due to infestication by *L. coffeella* moths is around 40% in Brazil. This can increase to as much as 80% in areas where the coffee-leaf-miner larvae are not controlled and the coffee plants become infesticated with the pest (REIS; SOUZA, 1996).

The biology of *L. coffeella* has been investigated by various authors (REIS; SOUZA, 1996; REIS; SOUZA; ZACARIAS, 2006; VEJA; POSADA; INFANTE, 2002). The *L. coffeella* moth measures 6.5 mm in wingspan and deposits its eggs on the adaxial coffee leaf surface. After the embryonic stage (4–6 days), the larva eclodes and perforate the upper epidermis of the leaf and penetrate

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Departamento de Ciências Exatas, Universidade Federal de Lavras, Caixa Postal 3037, 37200-000 - Lavras – MG, e-mail: scalon@dex.ufla.br (autor correspondente)

[2] Departamento de Ciências Exatas, Universidade Federal de Lavras, Caixa Postal 3037, 37200-000 - Lavras – MG, e-mail: gabriellacanapolis@yahoo.com.br

[3] Departamento de Ciências Exatas, Universidade Federal de Lavras, Caixa Postal 3037, 37200-000 - Lavras – MG, e-mail: mbavelar@yahoo.com.br

[4] EMBRAPA Café, EPAMIG/EcoCentro, Universidade Federal de Lavras, 37200-000 - Lavras – MG, e-mail: zacarias@epamig.ufla.br

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the mesophyll, feeding on palisade parenchyma cells. The lesions that form between the epidermis, also called galleries or tunnels or mines, have irregular margins, are pale yellow in color and later become brownish (REIS; SOUZA, 1996). The necrosed leaf surface reduces photosynthesis, since the flux of water, minerals, and organic matter is impaired. Nevertheless, the loss in production is mainly due to leaf loss, provoked by the increase in the level of ethylene, principally when the lesions are near the petiole (LE PELLEY, 1973). Subsequently, the larva leaves the mine and becomes a chrysalis that adheres to the abaxial leaf surface. The transformation of the chrysalis into a new moth completes the insect's life cycle (REIS; SOUZA, 1996).

The coffee-leaf-miner is usually controlled by insecticides, unfortunately this often leads to secondary outbreaks; additionally, the coffee-leaf-miner is becoming resistant to the chemical insecticides that are used to control the pest in Brazil. This in turn leads to a vicious cycle where more and more insecticide is used by the farmers to try to control the pest. This is neither healthy nor a good long term strategy for the control of coffee pests. Thus, Brazilian researchers have been continually making great efforts to improve the efficiency of pest control in organic coffee plantations by using biological mechanisms.

Before using an integrated pest management, it is important to carry out a sampling of insect populations. The apparent existence of spatial clusters of high incidence of many crop pests suggests that the spatial distribution of these pests might be influenced by environmental factors (BOEVE; WEISS, 1998). Thus, basic knowledge about the spatial behavior of the coffee-leaf-miner could potentially provide valuable information on where to release or conserve natural enemies in the context of biological control of the pest in organic coffee production and, therefore contributing to the efficiency of the development of organic cultivars.

The analysis of spatial distributions of insects in entomological studies, in general, involves collecting counts of the number of events in subsets of the study region. Then, indices such as the Fisher's variance-mean ratio and the Morisita's index of clumping can be used for testing complete spatial randomness (BOEVE; WEISS, 1998). Cressie (1993) points out that the reduction of spatial patterns to counts of the number of events in subsets and to one-dimensional index results in a considerable loss of information. Also, there is no consideration of subset location, so most of the spatial information is lost. Thus, the analysis of spatial distribution of insects requires methods which utilize more precise location information of the subsets.

Currently available technology, such as global positioning systems (GPS) and statistical methods for spatial data have opened up new ways to characterize, analyze and map insect distributions. Most applications of spatial statistics to insect ecology have been from forests and rangelands Entomology (KREBS, 1999; PERRY, 1998; SCHOTZKO; O'KEEFFE, 1989), with relatively few applications in agricultural systems (GARCIA, 2006; PARK; OBRYCKI, 2004). There are no studies applying specific statistical methods for spatial data to approach the problem of spatial heterogeneity of coffee-leaf-miner in organic coffee plantations. The aim of this work is to use spatial statistical methods (variogram) for characterizing the spatial distribution of the coffee-leaf-miner in a plantation of organic coffee (*C. arabica*) in formation during the annual peak population of the pest.

2 MATERIAL AND METHODS

Experimental

The study was carried out during the annual peak population period of the coffee-leaf-miner (September 2006) at the Cachoeira Farm, in Santo Antonio do Amparo, MG, Brazil (20°53' latitude S, 44°57' longitude W, altitude 1013 meters). The experimental area is almost rectangular. Its dimensions are 120 m by 90 m and the superficial expanse is approximately one hectare. The whole field was planted with two year old coffee (*Coffea arabica* L.) with 0.5 m between plants and 4.5 m between rows. An approximately regular grid was designed and situated over the experimental area. The grid was laid out in the field using the global positioning system (GPS), resulting in 35 sampling units areas with approximately 1.5 x 1.5 meters formed by one or two coffee trees. At each node of the grid, samples of ten leaves were taken from the canopy of the coffee plants and the number of mined leaves was counted.

Statistical methods

The phenomenon of this work is a random variable (number of mined leaves) obtained at 35 sampling units. A standard framework for the analysis of spatial data is to characterize the spatial dependence of the variable by using the spatial variation in distances between samples. Spatial dependence means that two sample values that are close to one another tend to be more similar than two values farther apart. Spatial dependence can be quantified on the basis of the semivariogram.

A semivariogram is a graph of the spatial dependence and plots half the squared difference of a sample pair (semivariance) against the distance between two points (BAILEY; GATRELL, 1995). Under the assumption of an intrinsically stationary process, the semivariogram captures the spatial covariance of the spatial process.

A natural estimator of the semivariogram is the empirical semivariogram value at distance interval *h* defined by

$$\hat{\gamma}(h) = \frac{1}{2\,|N(h)|}\sum_{i=1}^{N(h)}\{y(x_i) - y(x_i + h)\}^2$$

where *y*(*xi*) are measured sample values (number of mined leaves) at sample points *xi* and *N*(*h*) denotes the collection of pairs of observations whose spatial locations are separated by the distance *h*. In data that have been collected over an approximately regular grid, several pairs of points will be separated by the same value of *h* (BAILEY; GATRELL, 1995).

In order to determine whether or not the semivariogram functions depend on the sampling direction, we calculated the experimental semivariogram to each one of the four directions: 00 (E-W), 450, 900 (N-S) and 1350 and compared these semivariograms to the omnidirectional semivariogram (calculated by using the information of all directions). We used an angle of tolerance equal to $\pi/4$ (22.5 degrees) for calculating the directional semivariograms. We also used a randomization method for constructing confidence intervals for the empirical semivariograms. We adopted a number of randomizations equal to 999, which is the recommended minimum for obtaining the envelopes (DIGGLE; RIBEIRO JUNIOR, 2007).

We observe that the spatial dependence decreases for large distance classes, and bias may arise from the fact that only the observations from the edge of the sampled population can contribute to the estimates for larger distances. It is therefore customary to limit the description of the spatial structure to half the maximum distance between sampling units (CRESSIE, 1993). Thus, we adopted the maximum distance *h* equal to 60 meters for getting the experimental semivariogram.

All statistical analysis was carried out using the software R (R DEVELOPMENT CORE TEAM, 2009) and the contributed package geoR (RIBEIRO JUNIOR; DIGGLE, 2001).

3 RESULTS AND DISCUSSION

Several insect pests have been reported in coffee plantations, the most important being the coffee-leaf-miner (*L. coffeella).* One of the challenges in organic coffee production is the control of the pest outbreaks without the use of chemical pesticides. An attempt to control the pest in organic coffee is to use its natural enemies such as the predatory wasps (Vespidae) (VEJA; POSADA; INFANTE, 2006). It is accepted that interaction between predators and their prey can only be understood within a spatially explicit context. The lack of spatially explicitly, large scale spatial pattern field studies is identified as a major obstacle to the understanding of fundamental ecological processes (STEINBERG; KAREIVA, 1997). We investigated the spatial distribution of the coffee-leaf-miner in a relatively large scale plantation of organic coffee in formation.

During the first phase of the study, preliminary investigation was carried out on the number of mined leaves in order to check data consistency, presence of surface trend and identifying both outliers and the distribution where data came from. The proportion of mined leaves was equal to 0.84 ± 0.02 which is considered a high damage level. This number is expected in areas where the coffee-leaf-miner larvae are not controlled and the coffee plants become infested with the pest (REIS; SOUZA, 1996). That is the case of organic plantations. This high incidence is also expected in the driest months of the years since the coffee-leaf-miner does better in dry conditions. Under wet conditions, plants have very low infestation levels because of water entering the mine and drowning the larvae (VEJA; POSADA; INFANTE, 2006). September was one of the driest months of the year 2006 in the region where the experiment took place.

Figure 1 presents a two by two display showing locations of the 35 sampling points in the experimental

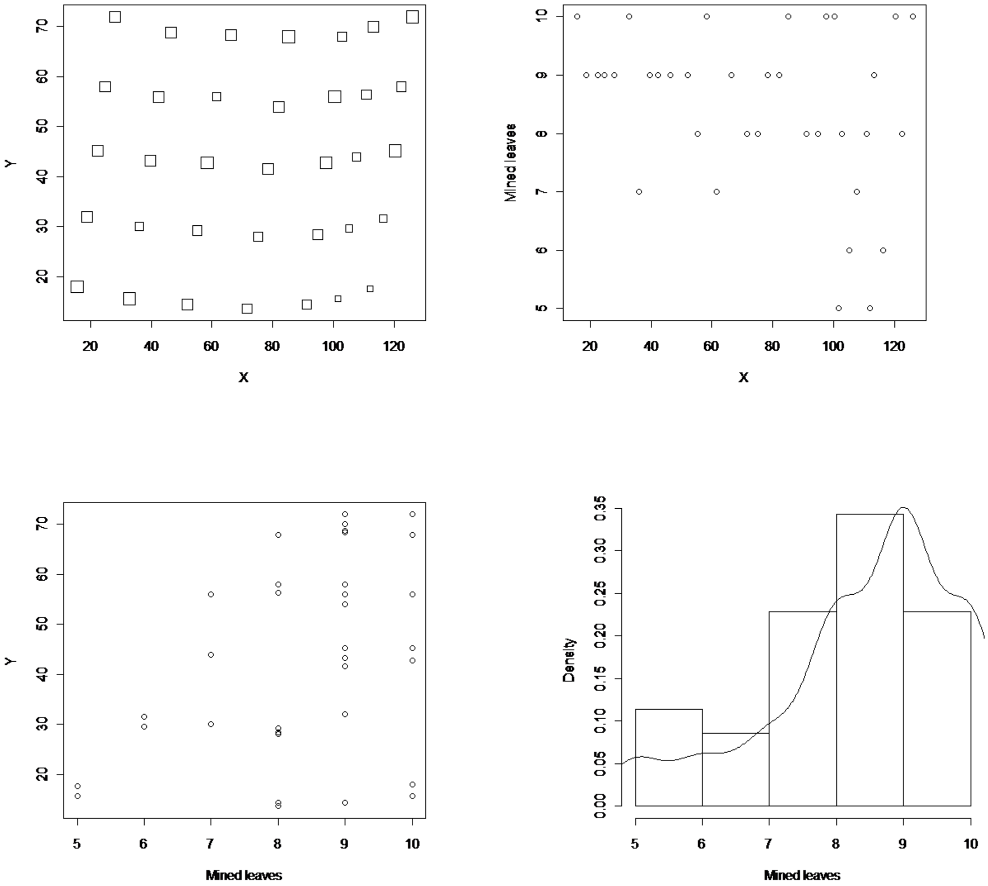

Figure 1 – Grid locations (top left), squares have areas proportional to the counts, data values against the coordinates (top right and bottom left) and histogram of the counts of mined leaves (bottom right).

area, where squares have areas proportional to the measured values of mined leaves, counts at each location against the coordinates, and a histogram of the counts of mined leaves.

The histogram of the 35 count values provides an idea about the distribution of the number of mined lives with mean = 8.43, coefficient of variation = 16.60 and skewness = -1.22 indicating low skew distribution to the left (negative skewness). Thus, the histogram indicates only a mild skewness to the left and does not suggest any obvious outliers.

The plot of the counts in relation to their location using squares with area proportional to the counts do not reveal neither apparent spatial outliers, that is, counts which appear grossly discordant with their spatial neighbors or spatial trends. The absence of spatial trend may be confirmed with the plots of the count data against the coordinates. It can be seen that the count data does not show dependence on latitude and longitude once the data does not show explicit preponderance of large (or small) counts towards some specific direction of the study region. Thus, a visual assessment of the exploratory analysis revealed no spatial dependence of coffee-leaf-miner in that month, so we then we proceeded with the calculus of the semivariograms. It is worth pointing out that the presence of normality of the variable is a desirable property, it is not a prerequisite for calculating the empirical semivariogram, and therefore no data transformation was carried out.

Diggle and Ribeiro Junior (2007) recommend the use of a randomization method for constructing confidence intervals for the empirical semivariogram in order to avoid subjective interpretation of the semivariogram for testing the null hypothesis of spatial independence. This means that for each simulation, data values are randomly allocated to the spatial locations. The empirical semivariogram is computed for each simulation using the same lags as for the semivariogram originally computed for the data. The envelopes are computed by taking, at each lag, the maximum and the minimum values of the semivariogram for the simulate data. The interpretation of these plots is straightforward. If the empirical semivariogram lies between the lower and upper envelops, it suggests acceptance of the null hypothesis of completely random spatial distribution (spatial independence) of the coffee-leaf-miner over the study region.

An important feature of an experimental semivariogram is the pattern of directional influences (or anisotropy) (CRESSIE, 1993). To determine whether or not the semivariogram functions depend on the sampling direction, Diggle and Ribeiro Junior (2007) advocate to calculate the experimental semivariogram to each one of the four directions: 00 (E-W), 450, 900 (N-S) and 1350 and compare these semivariograms to the omnidirectional semivariogram (calculated by using the information of all directions). Figure 2 shows semivariograms, generated for each one of the four directions, together with the two-tailed confidence intervals.

Figure 2 shows that variance between values of mined leaves does not present statistically significant changes with the distance, and thus the empirical semivariogram appears nearly horizontal, lying between the lower and upper envelops and shows no spatial dependence of coffee-leaf-miner. This behavior is

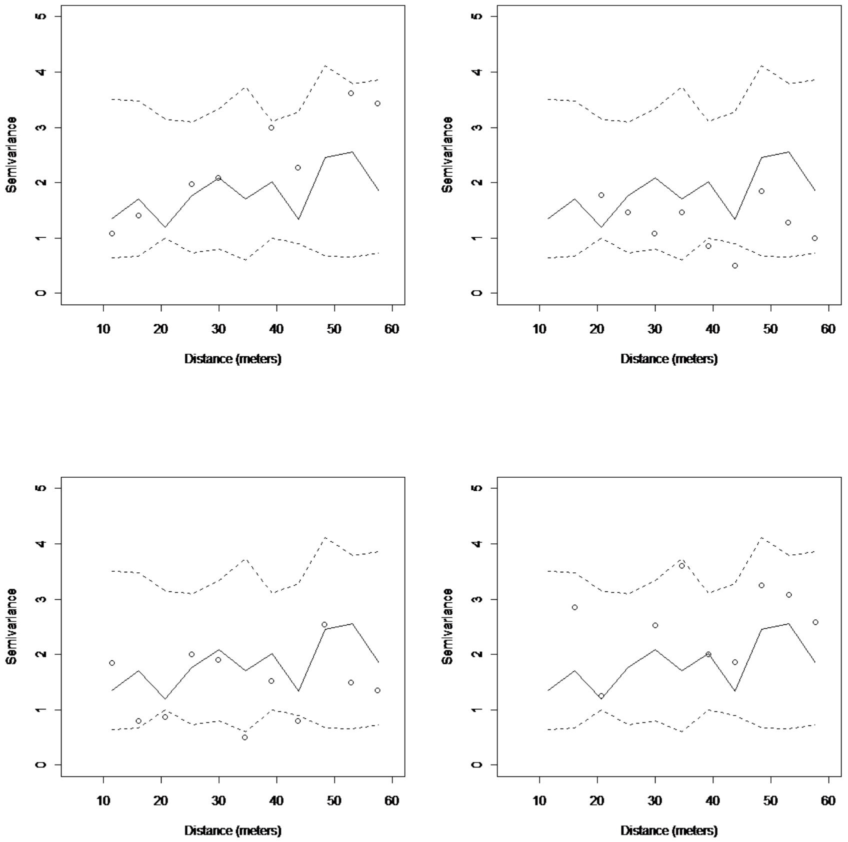

Figure 2 – Experimental omnidirectional semivariograms (solid line) for mined leaves. Dashed lines are the upper and lower envelopes from 999 simulations using randomization. Dots represent experimental semivariogram to each one of the four directions: 00 (top left), 450 (top right), 900 (bottom left) and 1350 (bottom right).

known as nugget model (BAILEY; GATRELL, 1995). We cannot also discriminate between the four direction models since they behave differently from each other but always revealing a pure nugget. Hence, we conclude that we have provided evidence that the coffee-leaf-miner does not present spatial structure during the annual peak population of the pest in an organic coffee field in formation.

These results could be seen as deceptive, since when ecologists seek spatial pattern (evidence of spatial non-randomness) they find it is the rule rather than the exception, because randomness implies the absence of behavior, and so it is unlikely a priori on evolutionary grounds.

It is possible that the evidence for non-randomness in the spatial distribution of the coffee-leaf-miner is due to the nature of the geostatistics. Perry (1998) argues that geostatistical approaches, developed for variables measured on continuous scales that display a stationary, stable covariance structure over a wide area, may not apply well to spatial pattern analysis for insect counts. However, many researchers have made use of these techniques with successes in the analysis of spatial patterns in Entomology (SCHOTZKO; O’KEEFFE, 1989) and agricultural systems (BACCA et al., 2006; GARCIA, 2006; PARK; OBRYCKI, 2004).

Nevertheless, we have applied other spatial statistical approaches based on counts of individuals per sampling units (Morisita’s index) for assessing the hypothesis of non-randomness in the spatial distribution during the annual peak of coffee-leaf-miner. Avelar (2008) has provided statistical evidence that the number of mined leaves does not present spatial structure while Scalon et al. (2011) has rejected the hypothesis of spatial randomness distribution in favor of an aggregated alternative for both new mines and preyed mines.

We advocate that the evidence for non-randomness in the spatial distribution of the coffee-leaf-miner is due to the nature of the variable used for characterizing the pest. We observe that in September, the driest month of the year 2006, the coffee-leaf-miner larvae were not controlled in the area, and therefore the organic coffee field was under a massive attack of the pest. In such situation of high incidence of mined leaves it is almost impossible to measure and detect the degree of non-randomness of the coffee-leaf-miner in the field.

3 CONCLUSIONS

The use of semivariograms showed that the coffee-leaf-miner population was randomly distributed in the field during the annual peak population. We expect that the results provided by this work may potentially provide valuable information in order to improve the knowledge of the spatial distribution of the coffee-leaf-miner in organic coffee fields.

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