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Sampling plan for the coffee leaf miner *Leucoptera coffeella* with sex pheromone traps

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Abstract

The population density of the coffee leaf miner *Leucoptera coffeella* (Guérin-Méneville & Perrottet) (Lep., Lyonetiidae) can be estimated using pheromone traps in coffee fields as male capture reflects this pest damage based on previous correlational study. However, the spatial distribution of pheromone traps and their density are necessary to optimize the sampling procedure with pheromone traps. Therefore, the objectives of the present study were to determine the pheromone trap density required per hectare to sample coffee leaf miner populations and to determine the spatial distribution of the males of this pest species. The males were sampled every 8 days in 12 consecutive evaluations. Taylor's power law and frequency distributions were used to recognize the distribution of the male capture data, which followed a negative binomial distribution. A common *K* was obtained, allowing the establishment of a single conventional sampling plan for the 12 fields investigated. The adjusted sampling plan requires eight traps in an area of 30 ha for a 25% precision error. Kriging-generated maps allowed the simulation of male captures for 8, 12 and 20 traps per 30 ha and the results were compared with those obtained with absolute sampling resulting in R^2-values of 0.30, 0.57 and 0.60 respectively. The traps were able to identify the more highly infested areas within the field and are a precise and efficient tool for sampling populations of *L. coffeella*.

Introduction

Integrated pest management programmes require precise and reliable methods to estimate density of pest insects and their natural enemies. The use of sampling plans allows the estimation of population density for pest control decision-making (Norris et al. 2003). Conventional and sequential sampling plans are frequently used to sample populations of the coffee leaf miner *Leucoptera coffeella* (Guérin-Méneville & Perrottet) (Lep., Lyonetiidae), the main coffee pest in Brazil and a key coffee pest throughout neotropical America (Pereira et al. 2007). These sampling plans require large amounts of leaves to determine the percentage of damaged leaves, demanding a great sample effort and leading to a delay in the decision-making process (Gravena 1983; Bearzoti and Aquino 1994; Souza et al. 1998; Vieira et al. 1999).

Traps with attractants, such as pheromones, have been successfully used for insect-pest sampling because they are a quick method and easy to use (Jones 1998). The use of this technique for the coffee leaf miner is possible because its sex pheromone is known and male capture with pheromone traps is correlated with pest damage (Francke et al. 1988;

Bacca 2006). The use of a sampling programme based on pheromone traps requires the assessment of the main factors affecting their capture, such as pheromone dose and persistence, trap prototype and its height, which were studied by Lima (2001). Trap density also affects insect capture and is thus regarded fundamental when using pheromone traps in sampling plans (Jansson et al. 1989). Nonetheless, the statistical analyses usually employed in developing sampling plans do not consider the spatial positioning of the samples, a shortcoming that can be solved using geostatistics (Liebhold et al. 1993).

Geographic information systems (GIS) have been used in several pest management studies. One of them is map building to visualize and monitor insect populations based on field sampling. Another application is the prediction of the population levels in non-sampled sites using interpolation to estimate a population based on neighbouring sample sites (Strother and Steelman 2001; Nansen et al. 2003). The objective of the present study was to determine the pest spatial distribution and to estimate the number of pheromone traps necessary to monitor populations of *L. coffeella* in a given area using a conventional sampling plan. Maps built through spatial interpolation techniques were used to validate the sampling plan.

Materials and Methods

Location and sampling

This study was carried out between August and November 2004, following up a previous investigation (Bacca et al. 2006) and encompassing the main period of infestation by *L. coffeella* in the region based on a preliminary 2-year survey at the site and previous information available (Souza et al. 1998). The coffee fields under study belonged to Da Terra Atividades Rurais Ltda, in the county of Patrocínio, state of Minas Gerais, Brazil. The experiment was carried out in a 30-ha area with 6-year-old coffee plantations (*Coffea arabica* cv. Tupi) with 2.5 m × 5 m spacing.

The sampling technique studied for capturing male insects used white delta traps (Delta, Biocontrole® Ltda, São Paulo, Brazil) and pheromone lures of 300 μg (racemic mixture of the synthetic pheromone 5,9-dimethylpentadecane, synthesized by Fuji Flavour Co. Ltd, Tokyo, Japan). The pheromone-baited lures were placed on white sticky pads (400 cm^2) and both were replaced every 4 weeks. The traps were suspended from coffee tree branches at 0.2–0.5 m. A total of 549 traps were deployed in grid arrays within the 30-ha area. These traps were divided into grids of 12 traps each, where the traps were spaced 2, 5, 10, 15, or 30 m apart. Each grid distance treatment was replicated six times and the distance between grids was of at least 50 m (fig. 1). During the last eight evaluations, 189 traps spaced 20 m apart were placed in the field to cover the whole area (fig. 1). All trap sampling sites were georeferenced using a global positioning system (GPS 12XL, Garmin, Olathe, KS, USA). Male capture with the pheromone traps was carried out weekly throughout the four months of the field study.

Determination of the number of traps and sampling plan

Taylor's power law was used to determine the theoretical distribution of the capture frequency of the data and consequently the most suitable model for estimating the number of samples (i.e. traps) required to establish the coffee leaf miner sampling plan with pheromone traps. If the value of the coefficient b of Taylor's power law is higher than 1, the data tend to better adjust to the negative binomial distribution; if $b \approx 1$, the best fit is usually provided by Poisson's distribution, while if $b < 1$, positive

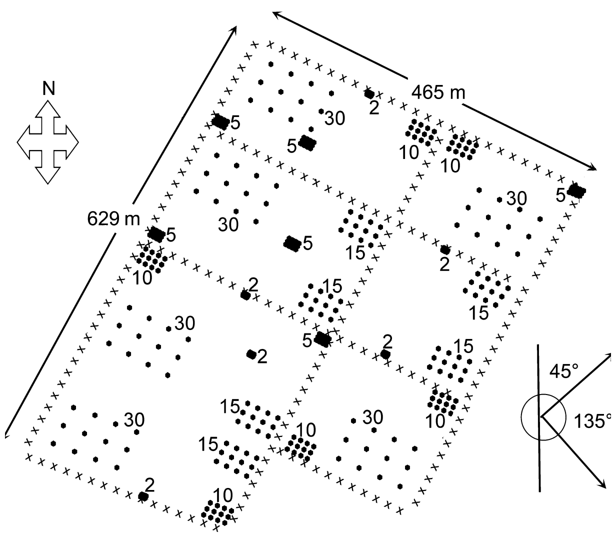

Fig. 1 Location of the 549 pheromone traps (circles and crosses) in the 30-ha area of coffee plantations. Six cluster of traps (circles) at each spacing where established in the area with interconnecting traps (crosses) linking the clusters. The crosses represent the 189 traps evaluated from the fifth to the 12th sampling date. The direction of 45° azimuth is in the same direction of the coffee rows, while the direction of 135° azimuth is perpendicular to the coffee rows.

binomial distribution usually provides the better fit (Young and Young 1998). The natural logarithm of the mean and the variance of each evaluation of male capture of *L. coffeella* were therefore determined to apply Taylor's power law:

$$\ln S^2 = \ln a + b \ln \mu$$

where a is Taylor's coefficient or sampling factor, b is the Taylor's b coefficient or the aggregation index, and μ is the population mean. The value of a is the intercept antilog and b is the slope of the estimated regression.

The most suitable distribution model was recognized and subsequently confirmed by calculating the expected frequencies based on the recognized model and comparing them with the observed frequencies using the chi-squared goodness-of-fit test (Pedigo and Zeiss 1996). Based on the distribution obtained, the existence of a common K-value (K_c) was determined to derive a dispersion parameter suitable to describe the variability observed among the coffee fields (Bliss and Owens 1958). The partial K-values (K_p) were initially calculated and their independence of the mean was tested by regression with the eventual estimation of K_c (Young and Young 1998). The K_c value was later used to calculate the number of traps required to establish the conventional sampling plan with *L. coffeella* pheromone traps using precision errors of 5%, 10%, 15%, 20% and 25% and the equation

$$NT = \frac{1}{C^2}\left(\frac{1}{\mu} + \frac{1}{k}\right)$$

where NT is the number of samples, C the allowed error, μ the population mean and k the parameter of the negative binomial model (Young and Young 1998).

Validation of the sampling plan

The maps of spatial distribution of *L. coffeella* male capture using 549 traps (absolute sample) in 30 ha were compared with the maps of male capture using the trap densities estimated for the sampling plan at 25%, 20% and 15% precision errors. Semivariograms were plotted for the first evaluation captures using the full set of 549 traps, which were used to build an ordinary kriging-generated map. The semivariograms were calculated, as previously described (Bacca et al. 2006), using data sets for the directions of 45° and 135° (azimuth) and the exponential regression model. Ordinary kriging is an interpolation technique of sampling data that estimates the values of data points spatially related. The formula used for kriging was:

$$\hat{Z}_{(X_0)} = \sum_{i}^{n} \left[\lambda_i \cdot z_{(Xi)}\right]$$

where $\hat{Z}_{(X_0)}$ is the semivariogram estimated value for a non-sampled site, n represents the total number of traps, $Z_{(Xi)}$ is the capture value of each trap, and λ_i is the weight of each $Z_{(Xi)}$ calculated from the semivariance matrix (Liebhold et al. 1993).

The kriging technique is a more exact method for interpolation mainly because it interpolates site-specific values considering their spatial dependence and anisotropy (i.e. inequality between different directions in the distance grids used), and more accurately describes the spatial structure of the data generating more valuable information on the error distribution (Rossi et al. 1994). However, the use of this technique requires several sampling sites to prevent the formation of erratic patterns in the semivariogram, what limits map-building from the kriging technique. This was the reason why map-building with only the number of traps estimated for the sampling plan was not possible. In this case the maps were built using the spatial interpolation of the inverse weighted distance, where the capture of neighbouring traps to a central trap are assumed to be related by their proximity (Brookers 1991). The neighbouring traps were weighted as a decreasing function with the increasing distance following the equation:

$$\hat{Z}_{(X_0)} = \frac{\sum_{i=1}^{n} Z_{(Xi)}/h_{ij}^{\beta}}{\sum_{i=1}^{n} 1/h_{ij}^{\beta}}$$

where $\hat{Z}_{(X_0)}$ is the estimated value (by interpolation) for the non-sampled site, n is the total number of traps, Z_{Xj} is the amount of captures for each trap, h_{ij} is the distance between sampled and interpolated sites and β is the distance weighted (2 in the present case) (Brookers 1991). The maps were generated from the captures of 8, 12 and 20 traps, systematically distributed to cover the whole area. Half of the traps were placed at the edges of the experimental field to detect dispersal from neighboring areas and the remaining half of the traps were placed within the experimental field (fig. 1).

Linear regressions ($P < 0.05$) were generated to estimate the capture precision relating the estimated capture obtained with the interpolation technique above referred and the capture estimated by ordinary kriging from the 549 pheromone traps placed in the

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area. This procedure was carried out for the trap densities of 8, 12 and 20 traps in the 30-ha area.

Results

Number of traps and sampling plan

The regression between the natural logarithm of the variance and the natural logarithm of the mean, called Taylor's power law, were significant for the whole group of 12 evaluations of male capture of *L. coffeella* (F = 132.59, n = 12, P < 0.0001) (fig. 2). The *b* coefficient of Taylor's power law was significantly higher than one by the Student's t test (P < 0.05) for male capture (*b* = 1.89, t = 5.43, n = 12), indicating that the data from male capture of *L. coffeella* probably follows the negative binomial distribution.

The male capture data of *L. coffeella* were subsequently adjusted to the negative binomial distribution showing a good fit in 11 of the 12 dates where male capture was evaluated, unlike what happened with the two alternative distribution models, Poisson and positive binomial (table 1). The data sets were therefore adjusted to a common aggregation parameter (K_c) of the negative binomial distribution. The slopes of K_c were significant (P < 0.05), but their intercepts were not (P > 0.05) indicating the existence of a common aggregation parameter (K_c) for the capture of *L. coffeella* males with pheromone traps (table 2). The K_c thus obtained allowed the establishment of a sole sampling plan for all of the coffee fields under the 30-ha area under investigation. The required number of traps for sampling males of *L. coffeella* stabilized at the 25% precision error (fig. 3). At his precision level, the conventional sampling plan requires eight pheromone traps for sampling the 30-ha area. Increasing precision to the levels of 20% and 15% requires 12 and 20 traps in 30 ha.

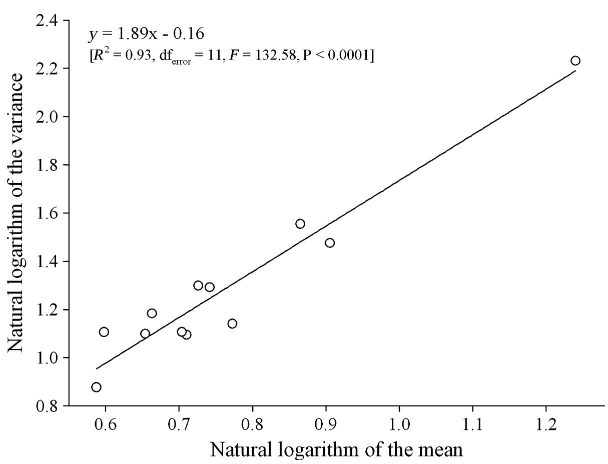

Fig. 2 Regression curve between the natural logarithm of the variance and the natural logarithm of the mean number of males captured of *Leucoptera coffeella* to obtain the coefficients of Taylor's power law.

Validation of the sampling plan

The maps of spatial distribution of the male capture by the pheromone traps also showed an aggregate distribution (fig. 4). Isolines and different tones in the maps represent grouped areas with similar captures ranging from 0 to 80 males per trap. The map generated with the full set of 549 traps (representing the absolute sampling) showed higher captures and therefore higher infestations in the bottom and lower left corner of the map with captures around 40 males per trap (fig. 4a). The same areas of higher capture were also recognized in the maps generated with 8, 12 and 20 traps (fig. 4b–d), but lower number of traps resulted in an overestimation of the capture. The maps given in fig. 4a,b are slightly different from those presented in fig. 4c,d because of the higher number of traps used on the first set of maps, allowing slightly greater extrapolation of the edges of the kriging-generated maps. Lower trap densities necessarily lead to reduced limits of the kriging-generated maps that more closely follow the outline of the sampling points delimiting the field (fig. 4c,d). However, the lower density of pheromone traps combined with the placement pattern used in the present study (i.e. half of the traps around the edge of the field) allowed accurate recognition of the male capture throughout the field.

The linear regressions carried out between the capture estimated with the kriging technique (with 549 traps) and the capture estimated using the inverse weighted distance with the trap densities estimated by the sampling plan were all significant, but the precision and reliability of the captures decrease when the trap density decreases from 20 to eight traps (fig. 5).

Discussion

Sampling plans based on pheromone traps require the presence of a significant functional relationship between pest capture and pest damage to allow management decisions based on trap captures (Wall 1989; Jones 1998). Such relationship was recognized by Bacca (2006) in studies with male capture of

Table 1 Goodness-of-fit chi-squared (χ^2) test for observed and expected frequencies of male capture of *Leucoptera coffeella* following the frequency distribution models of Poisson, negative and positive binomial

			Poisson distribution		Negative binomial distribution			Positive binomial distribution	
Coffee fields	μ	S^2	$\chi^2_{calculated}$	Degrees of freedom	$\chi^2_{calculated}$	Degrees of freedom	k	$\chi^2_{calculated}$	Degrees of freedom
1	17.38	170.27	1377.20*	52	63.60^{ns}	51	1.98	1618.46*	51
2	7.33	35.85	182.83*	27	39.77^{ns}	26	1.88	253.79*	26
3	8.04	29.94	1215.38*	26	30.29^{ns}	25	2.3	815.45*	25
4	5.13	12.42	238.27*	17	13.85^{ns}	16	3.62	347.41*	16
5	3.87	7.53	32.31*	12	16.94^{ns}	11	4.08	60.46*	11
6	5.32	19.90	138.43*	16	12.69^{ns}	15	1.94	200.43*	15
7	5.93	13.82	72.64*	16	11.08^{ns}	15	4.45	132.26*	15
8	5.52	19.58	154.87*	17	24.84^{ns}	16	2.166	291.77*	16
9	5.06	12.78	84.05*	15	13.68 ^{ns}	14	3.31	212.72*	14
10	4.61	15.25	165.77*	15	24.91*	14	1.99	149.74*	14
11	4.51	12.56	101.90*	15	17.64^{ns}	14	2.52	105.74*	14
12	3.96	12.75	49.10*	14	16.10^{ns}	13	1.79	81.34*	13

Estimated K_{common} = 2.17.
*Significant at P < 0.05.
^{ns}Non-significant at P < 0.05.
Degress of freedom = $k - 1 - m$, where k is the number of classes and m is the number of parameters in the model.

Table 2 Analysis of variance for verification of the common dispersion parameter (K_c) of the negative binomial distribution of the male capture of *Leucoptera coffeella* in 12 coffee fields

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	$F_{calculated}$
Slope $1/K_c$	1	5114.85	5114.85	305.58*
Intercept	1	53.79	53.79	3.21^{ns}
Error	8	133.90	16.74	

*Significant at P < 0.05.
^{ns}Non-significant at P < 0.05.

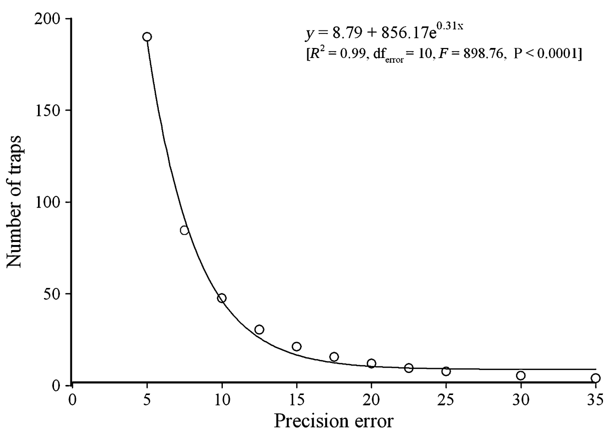

Fig. 3 Number of samples (traps) required for sampling males of *Leucoptera coffeella* under different precision errors of the conventional sampling plan using pheromone traps in coffee fields.

L. coffeella using pheromone traps carried out for 20 months in two coffee-producing regions in Brazil. As a consequence, the present study was carried out to determine the pheromone trap density required per hectare to sample coffee leaf miner populations and to determine the spatial distribution of the males of this pest species, optimizing its sampling procedure with pheromone traps.

The capture of males of *L. coffeella* with pheromone traps followed the negative binomial frequency distribution model as earlier observed in studies of leaves mined by this species (Villacorta and Tornero 1982; Santana et al. 1993; Oliveira 2003). This is no surprise, as the negative binomial is the most frequent frequency distribution model observed in insect populations (Taylor 1984). However, the distribution pattern of a given species may vary with the region, crop species and phenology, plant location and the pest management programme used. The pattern of spatial distribution of an insect species may also change during its development. The effect of extreme environmental conditions on insect activity can be regarded as largely an all or nothing response, so that marked activity thresholds exist (Taylor 1963). In addition, release rates of pheromone are influenced by environmental factors that can also affect flight into the crop (Wall 1989).

The aggregate pattern of distribution in insects suggests that the individuals are concentrated in

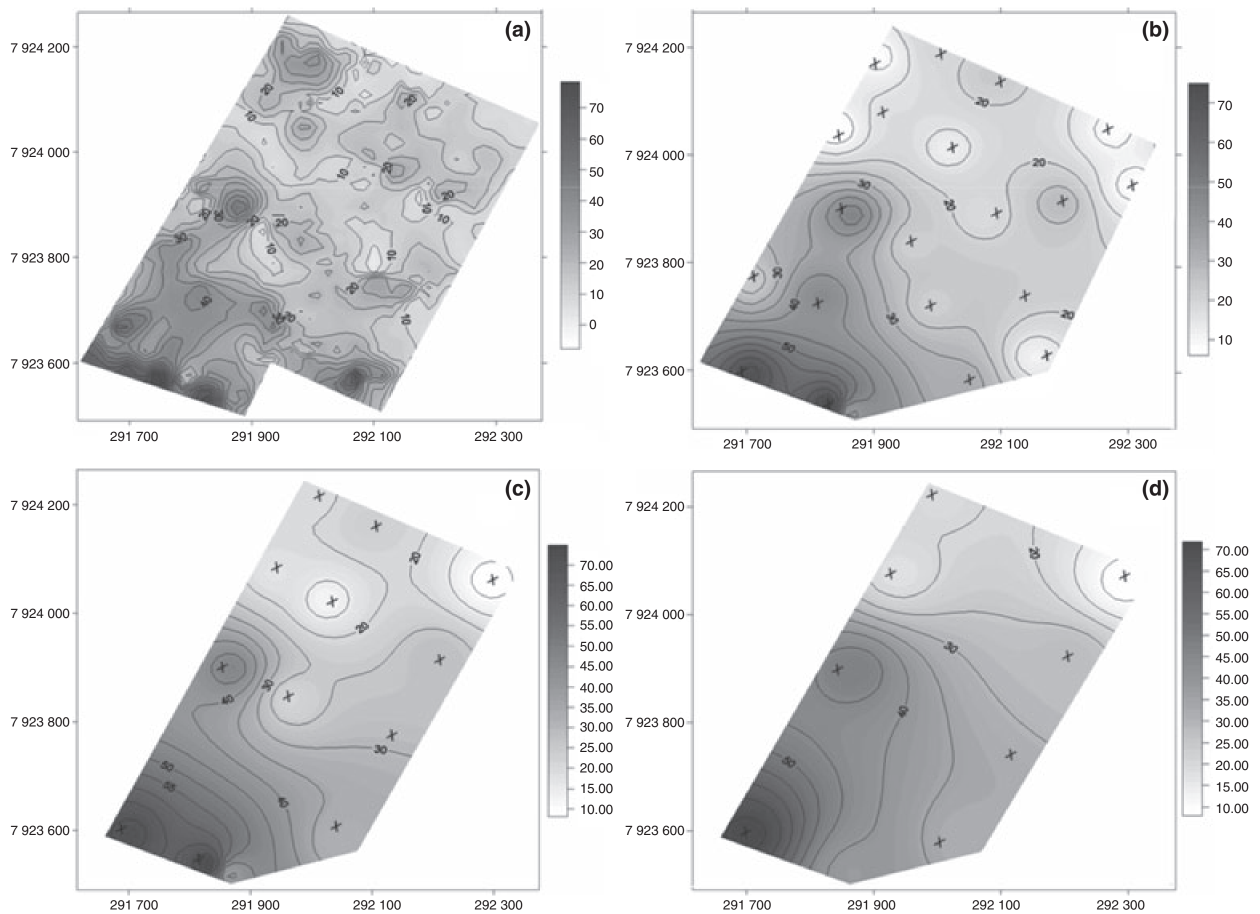

Fig. 4 Maps of the estimated captures of males of *Leucoptera coffeella* generated using 549 traps and kriging (a), and using either 20 (b), 12 (c) or 8 (d) traps and interpolation by the inverse of the weighted distance. The placement of the pheromone traps in map (a) is indicated in fig. 1, while the symbol 'x' indicates the location of each pheromone trap within the experimental field in the maps (b), (c) and (d).

more favourable parts of the habitat because of their higher resource availability, environmental heterogeneity, and the insect species aggregation behaviour for mating or defence for instance (Taylor 1984). The availability of plant defensive chemicals may also affect the development or reproduction of herbivore insects interfering with their spatial distribution (Karban and Baldwin 1997). An example is the content of caffeine and *p*-cymene in coffee leaves mediating egg-laying by *L. coffeella*, as observed by Magalhães (2005), which may contribute to the pattern of spatial distribution of this species.

Inferences on the type of spatial distribution of insects are usually drawn from the relationship between the mean and variance followed by fitting the data to mathematical distributions (Young and Young 1998). Despite this, the frequency distributions do not always reflect the spatial distribution of insect populations in the field (Young and Young 1998), as observed by Barrigossi et al. (2001) studying the egg distribution of the Mexican bean beetle *Epilachna varivestis* Mulsant (Col., Coccinellidae). Geostatistical procedures are able to circumvent this problem as they can be carried out to analyse the spatial relationships among individual insects of a species (Liebhold et al. 1993). The aggregate pattern of spatial distribution reported for *L. coffeella* was confirmed with the geoanalysis (for the semi-variogram data, see Bacca et al., 2006) and clearly shown in the maps generated.

The presence of a common aggregation parameter in the negative binomial distribution of male capture

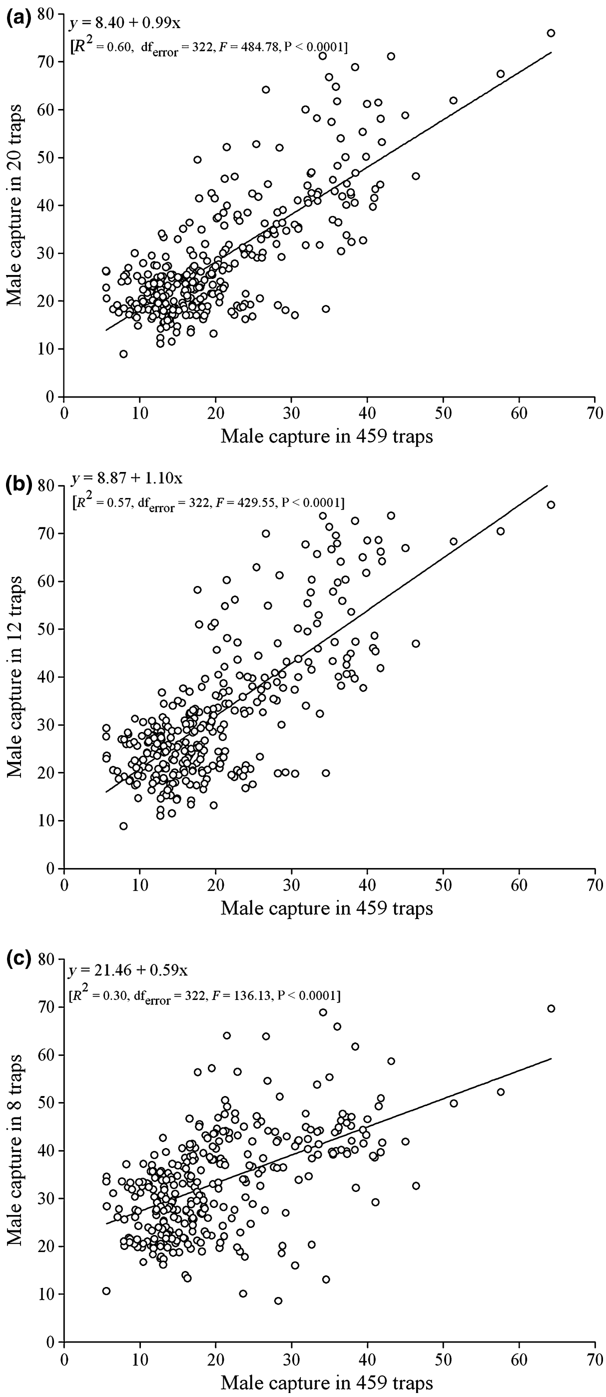

Fig. 5 Relationship between the estimated capture of males of *Leucoptera coffeella* using spatial interpolation by the kriging method using 549 traps and the interpolation by the inversed of the weighted distance using 20 (a), 12 (b) and 8 (c) traps.

of *L. coffeella* allowed the establishment of a single conventional sampling plan for all of the coffee fields assessed and using a precise number of traps – 8 per 30 ha (or 1 trap per 4 ha). The use of such trap density is feasible and allows the sampling of large areas at a low cost. The procedure of sampling by pheromone traps using the developed sampling plan allows the sampling, processing and decision-making for this insect pest control within a single day. This is a great improvement compared with the 100 to 200 leaves that need to be collected per coffee field using the conventional sampling plans earlier suggested by Gravena (1983) and Souza et al. (1998). Sequential sampling plans later developed decreased the number of samples to five to 24 per coffee field depending of the infestation intensity (Bearzoti and Aquino 1994; Vieira et al. 1999; Oliveira 2003), which is comparable with the conventional sampling plan proposed here using pheromone traps for capturing males of *L. coffeella*. However, the sequential sampling plans developed for *L. coffeella* were not subjected to field validation considering varying coffee field sizes, limiting their applicability. In contrast, the present sampling plan using pheromone traps not only considers the distribution of the pest species, but also the spatial interference between the samples.

The required trap density to monitor *L. coffeella* reported here (1 trap/4 ha) was further supported by the findings of Bacca et al. (2006) on pheromone trap interference. Adults of *L. coffeella* affected by the pheromone plume may disperse from the site where it is damaging the crop, which could compromise the precision of spatially detecting the main areas of infestation. However, a single pheromone trap of *L. coffeella* detects the presence of males of this species in an area of nearly 4000 m^2 (Bacca et al. 2006), minimizing the relevance of the mobility of adult males of this species in compromising the efficiency of pheromone traps and allowing the recognition of coffee-infested areas. Here, the traditional methods commonly employed to develop sampling plans were aided by the use of semivariograms and spatial interpolators in estimating the trap density required for sampling the coffee leaf miner and to validate such estimation. The trap density of 1 trap/4 ha resembles the density recommended for monitoring males of the codling moth, *Cydia pomonella* (L.) (Lep., Olethreutidae), in walnuts, but in peach orchards even lower trap density is required (Rield 1980; McNally and van Steenwyk 1986).

The spatial interpolation used in this study was helpful in simulating the results generated in the conventional sampling plan using pheromone traps for *L. coffeella* and validating the plan. The predictions from the captures generated with 8, 12 and 20 traps provided a good approximation to the captures obtained with 549 traps, although reducing the number of traps weakens the prediction and reliability of the captures. However, the lower the number of traps, the cheaper is the sampling without substantially compromising its reliability.

The maps generated by geostatistical techniques proved useful in identifying the main areas of infestation of the insect pest, which will be important for targeting the insecticide spraying reducing the pest control costs and the environmental problems related to this control method. For instance, Weisz et al. (1996) were able to reduce 30–40% of the insecticide spraying commonly used to manage the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col., Chrysomelidae), in the US using such maps.

In summary, the sampling plan using pheromone traps developed for monitoring populations of the coffee leaf miner *L. coffeella* was fast and precise reflecting the pest infestation in the coffee field. In addition, the sampling plan allows the identification of the main areas of infestation with high precision and consequently the control effort can be properly directed to these more heavily infested areas increasing its efficiency and decreasing its costs and non-target impact.

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