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Contributions of Monte-Carlo test procedures for the study of the spatial distribution of the European Vine Moth, *Lobesia botrana* (Lepidoptera: Tortricidae) in European vineyards

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Abstract. The efficiency of Monte-Carlo procedures to test some hypotheses about the spatial patterns of larvae and damages of *Lobesia botrana* was studied. Two hypotheses were tested to detect spatial heterogeneity and spatial dependence. The most practical implication is to provide an efficient sampling scheme. The study of the relationship between spatial patterns and grape availability was required to explain scales of spatial heterogeneity and population dynamics studies were needed to relate it to oviposition behavior. It was tested through a third hypothesis. We adapted Monte-Carlo simulation procedures for the analysis of exhaustive count data obtained from regular grids delimited within each of two vineyards. Statistical analyses were based on count permutations and on count redistributions according to the hypotheses which were tested. Indices of aggregation and autocorrelation statistics were used. The hypotheses that we tested at different scales were random distribution of the infestations (H_R), independence of vine stock (or groups of k vine stocks) infestation (H_I) and independence between vine stock infestation and grape availability (H_G). Monte-Carlo tests revealed the same spatial patterns for larvae and damages. We detected different spatial patterns. The implications for sampling were that sample unit could be an individual stock and that sampling along a row could not be used to estimate population density in the vineyard. Results showed that infestation of a given stock depended on grape availability on this stock and on neighboring vine stocks.

INTRODUCTION

Lobesia botrana (Denis and Schiffermüller) is considered a major pest of vineyards in European countries (Roehrich & Schmid, 1979; Roehrich & Boller, 1991). According to the climate, the European Vine Moth (EVM) has two generations (Austria, Germany, Switzerland) or three generations (South of France, Mediterranean countries). The EVM damages vary from year to year and from vineyard to vineyard, depending on vine phenology and moth phenology. The first generation larvae feed on grape flowers making silked flower clusters. The second generation larvae feed on young green berries while the third generation larvae damage maturing berries. The injury threshold for the first generation varies from 15 to 100 larvae per 100 grape flowers depending on vine ability to compensate for flower suppression which can be high and is related to vine variety (Roehrich & Schmid, 1979). The damages caused by larvae of second and third generation are indirect because they increase vine attack by *Botrytis cinerea*. In European countries, the action threshold varies from 2 to 20 larvae per 100 grapes, according to rainfall.

Pheromone trapping provides information on moth phenology but is not a reliable indicator of EVM density in vineyards (Roehrich & Boller, 1991). In these conditions, Integrated Pest Management programs require performing tools for sampling moth density in vineyards. Estimating larval population density in a vineyard requires to have defined how much precision is needed and a sampling technique which minimizes sampling time and gives the pertinent information. Some hypotheses are required before defining the sample unit and the procedure by which the sample may be selected. Independence between sample units must be verified to define the sample unit, the sample must be representative of the population to be sampled, and redundant information should be avoided to minimize sampling time. Information about the spatial distribution of the pest is needed to answer these questions. On the other hand, the description of the spatial pattern of the pest and especially the scales at which spatial heterogeneity occurs is required to improve control by mating disruption (Schmitz, 1992). The efficiency of this method seems to be influenced by the local spatial distribution of the moth (Schmitz, 1992). Environmental factors which might influence the spatial distribution of the pest need to be pointed out. Taking them into account could improve pest control and studies on the oviposition behavior in the populations.

Very little is known about the spatial distribution of *L. botrana.* Geier et al. (1953) found that the distribution of larvae of both *L. botrana* and *Eupoecilia ambiguella* (Hbn.) was not random: variability was greater inside groups of four neighboring stocks (two adjacent stocks on a planting row on two neighboring planting rows) than between groups of the same size. These results concerned two moth species, *E. ambiguella* being highly dominant. On the other hand, no indication was given on population distribution at other scales.

A wide variety of statistical methods have been used to characterize spatial distributions. Some of them ignore the spatial position of the sample units and are based on the estimates of distribution parameters as, for example the parameter of aggregation of Taylor's power law (1961), Lloyd's mean crowding (1967), or the parameter k of the negative binomial distribution (Johnson & Kotz, 1969). These parameters are of practical importance for the development of sampling methods but are difficult to interpret from an ecological point of view because the spatial integrity of the observations is not maintained. On the other hand, with such methods, it is assumed but generally not verified, that the sample units are independently infested. The purpose of this study was to show how Monte Carlo test procedures (Barnard, 1963) could be valid and useful to answer to our questions about spatial patterns. The procedures that we performed were based on indices of aggregation and spatial autocorrelation statistics, calculated from exhaustive counts of damages and larvae of the first and second generations of *L. botrana* in vineyards.

MATERIAL AND METHODS

Monte-Carlo tests

Besag & Diggle (1977) adapted the original Monte Carlo tests (Barnard, 1963) to spatial data analyses. These tests are based on the simulation of the tested hypothesis, for example that adjacent units are infested independently of each other. We can choose a statistics u to test the hypothesis H_0. The observed data set gives a calculated value u_{obs} of u. The hypothesis H_0 is simulated s–1 times, and for each simulated run, the statistics u is calculated. Thus, we obtain s–1 values of u: $u_1,\ldots\ldots,u_{s-1}$. If these values and u_{obs} are ordered such as $u(1),\ldots,u(s)$, we have under the hypothesis H_0:

$$P(u_{obs} = u(i)) = \tfrac{1}{s} \qquad (i = 1,\ldots,s)$$

Therefore, the rank of u_{obs} ($r(u_{obs})$) may be used to construct an exact test of the hypothesis H_0. It is based on the property:

$$P(r(u_{obs}) \leq k) = \tfrac{k}{s} \qquad \text{with } k = s\alpha$$

Type I error is α and the number of simulations needed to have a good power of the test is such as $s\alpha \geq 5$ (Hope, 1968). Thus, if $\alpha = 0.05$, the number of simulations must be >100. If s = 200, the rank of u_{obs} which leads to rejecting of the hypothesis H_0 is <6 or >195 for a two-tailed test.

This kind of procedure leads to valid tests because it is not necessary to know the exact distribution function of the statistics which is often constrained to conditions such as mean >5 or to approximations which are not always valid. On the other hand, the variety of hypotheses that can be tested can be large as the only condition is that we can simulate them.

Sampling data

Data were collected in 1996 in two naturally infested and untreated vineyards in Preignac (France) and in Wachenheim (Germany). In Preignac, the observations were carried out on the first generation of the moth (G1P) during the first week of June (3rd to 6th June), and on the second generation (G2P) during the last week of July (23rd to 30th July), while they concerned the first generation in Wachenheim (G1W) from 24th to 26th June. In Preignac, the vineyard was 15 years old and consisted of 6,300 vine stocks of the Sauvignon variety planted per hectare. Its area was 2.5 ha, and the intervals were 1.60 m between vine stock rows and 1 m between vine stocks on a row. In Wachenheim, the vineyard was 15 years old planted with 3,790 vine stocks per hectare of the Riesling variety. The area was 5 hectares and the intervals were 2.2 m between rows and 1.2 m between stocks on a row.

The observations were carried out exhaustively on subplots of 4 adjacent rows of 84 adjacent vine stocks for G1P (336 vine stocks), 3 other adjacent rows of 84 adjacent vine stocks for G2P (252 vine stocks), and 4 adjacent rows of 80 adjacent vine stocks for G1W (320 vine stocks). The vineyards consisted of about 90 vine stocks per row, and the subplots were located at the centre of the vineyards to avoid edge effects.

The observations were carried out after egg hatching and before the leaving of the oldest larvae for pupation in the foliage or the trunks. For the first moth generation (G1P and G1W), the number of damages was the number of larval nests. For the second generation of the moth (G2P) the number of damages was the number of foci with foraged berries, a foci being an isolated or a group of foraged berries. The number of damages per grape, for all the grapes of each vine stock in the subplot, was determined directly in the vineyard. Larval nests or foci with foraged berries were brought back to the laboratory and were dissected to determine the number of larvae. In all locations, we calculated the number of larvae per stock, the number of damages (larval nests or foci with foraged berries) per stock and the number of grapes per stock.

Data analysis

For data analysis we considered that each subplot in the vineyard was a regular grid consisting of 4 (G1P, G1W) or 3 (G2P) rows and 84 (G1P, G2P) or 80 (G1W) vine stocks.

To study the scales of pattern, we tested the hypotheses described later, at different sizes of unit by associating adjacent vine stocks into successively larger units of size $k = r \times c$ vine stocks, r adjacent vine stocks on the same row and c adjacent vine stocks on different rows. When $r = c = 1$, the unit is the vine stock. This method was first described by Greig-Smith (1952) for the study of plant community structures.

The number of simulations calculated according to Hope's (1968) recommendations, was 200, and $\alpha = 0.05$. Simulations were conducted using programs (Badenhausser, 1993) developed with Splus language (Venables & Ripley, 1994). We tested three hypotheses for a given scale k. Hypotheses H_R and H_I were tested in order to define a sample unit and the procedure by which sample could be collected to estimate population density at a given scale. Spatial variability and the scales at which it occurred were also described by testing these hypotheses. The independence between stock infestation and grape availability was tested through hypothesis H_G at different scales.

H_R: hypothesis of random distribution of the total number of larvae, $N = \sum_{i=1}^{n} z_i$, or damages, $D = \sum_{i=1}^{n} y_i$, observed on the n units. The hypothesis was simulated by redistribution of N insects or D damages independently of each other in the n units. To illustrate the simulation, let us take the following observed count data set (S1):

$$\begin{matrix} 0 & 3 & 0 & 2 \\ 0 & 0 & 2 & 0 \\ 1 & 1 & 1 & 1 \\ 1 & 2 & 0 & 0 \end{matrix}$$

A possible simulated run under H_R at scale 1 × 1 could be:

$$\begin{matrix} 0 & 2 & 0 & 0 \\ 0 & 0 & 2 & 0 \\ 1 & 0 & 1 & 2 \\ 2 & 2 & 1 & 1 \end{matrix}$$

Two statistics were chosen. The index of dispersion (Pielou, 1969) tests H_R against regularity or against aggregation. Regularity can be defined as a spatial pattern in which the possibility of any individuals occurring at distances of less than d apart is null, i.e. for which the individuals in the population are equidistant from each other (Upton & Fingleton, 1988). It could be produced by a territorial behaviour, which when observed, concerns generally a small area. Random distributions are not frequent in insect populations. Aggregation is more frequent due to environmental heterogeneity or to insect behavior. I_D is calculated as

$$I_D = \frac{\sum_{i=1}^{n}(x_i-\bar{x})^2}{(n-1)\bar{x}} \text{ with } \bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

where x_i is the count (larvae or damages) on unit i.

The local variance provides information about the difference between two adjacent units. It was calculated when units were neighbors on the same row (V_s) and when units were neighbors on different rows (V_r). If counts are randomly distributed, the differences between two adjacent units are small (but not too small), while aggregation is detected when the differences between two adjacent units are high,

$$V=\sum_{i=1}^{n-1}\frac{(x_i-x_{i+1})^2}{x_i+x_{i+1}}$$

Aggregation does not mean that counts in the units are correlated or independent of each other, but that some units have no individuals and some have a lot.

H_I: Hypothesis that all count permutations (insects or damages) have the same probability of occurring, i.e. vine stocks or group of vine stocks are infested independently of each other. The hypothesis was simulated by random permutation of the n units. The counts or damages per unit were maintained but their spatial integrity was not. To illustrate the simulation, we can take data set S1. A simulated run under H_I at scale 1 × 1 could be:

1 1 1 0
2 0 0 1
0 3 0 0
1 0 2 2

If the pattern scale is k = 2 × 2 a possible simulated run under H_I from data set S1 could be:

2 5
3 4

Moran's indices (Cliff & Ord, 1973) characterize the autocorrelation between neighboring units

$$I_M = \frac{n\sum_{i\neq j}^{n}(x_i-\bar{x})(x_j-\bar{x})w_{ij}}{\left(\sum_{i\neq j}^{n} w_{ij}\right)\sum_{i=1}^{n}(x_i-\bar{x})^2}$$

where $w = (w_{ij})$ is the weighting matrix which gives the possibility to have different weights according, for example, to the proximity of the neighbors. In our case, w_{ij} = 1 if i and j are defined as neighbors and w_{ij} = 0 if not. We calculated three indices: I_{Ms} when units i, j were neighbors on the same row with $j = i + 1$, I_{Mr} when units i, j were neighbors on different rows with $j = i + 1$, and I_{M4} when we considered the two nearest neighbors on the same row and the two on different rows.

H_G: Hypothesis that a vine stock (or group of vine stocks) is infested independently of the number of grapes of the stock (or of the group of stocks) and of its neighbors. The hypothesis was simulated by random permutation of the number of larvae in the n units. The numbers of larvae and of grapes per unit were maintained, but their relative spatial position was not maintained. We calculated the correlation $r(x_i, y_i)$ between the number of larvae per unit x_i and the number of grapes of the same units y_i, and the covariance $cov(x_i, y_{i-1} + y_{i+1})$ between the number of larvae per unit x_i and the number of grapes of the nearest neighboring units on the row y_{i-1} and y_{i+1}.

TABLE 1. Statistical description of larvae and damage counts per vine stock in three vineyards. I_D, index of dispersion, SD, standard deviation.

	G1P		G2P		G1W	
	Larvae	Damage	Larvae	Damage	Larvae	Damage
Mean	1.67	2.88	1.04	1.48	3.42	5.68
SD	1.98	3.42	1.39	1.99	3.01	4.78
I_D	2.35	4.07	1.86	2.69	2.65	4.02
Number of stocks with:						
0	122	104	127	110	48	33
1	79	52	55	55	43	27
2	50	36	32	30	55	46
3	33	38	18	21	53	24
>3	52	106	20	36	121	190
% infested, damaged	64	69	49	56	85	90

H_R, H_I were tested for each of the vineyards at different scales $k = r \times c$, with r = 1, 2, 4, 5 (G1W) or 6 (G1P, G2P), 10 (G1W) or 12 (G1P, G2P), 20 (G1W) or 21 (G1P, G2P), 40 (G1W) or 42 (G1P, G2P), and c = 1, 2, 3 (G2P) or 4 (G1P, G1W). When c = 3 or 4, Vr and I_{Mr} were not calculated.

H_G was tested for each of the vineyards at different scales: $k = r \times c$, with r = 1, 2, 4, 5 (G1W) or 6 (G1P, G2P), 10 (G1W) or 12 (G1P, G2P), and c = 1.

RESULTS

Spatial pattern of larvae and damages in vineyard G1P

The mean number of larvae per vine stock was 1.67 with 64% of infested stocks (Table 1), while the mean number of damages per vine stock was 2.88 which corresponded with 69% of damaged stocks. This could be explained by the fact that one larva can make more than one damage or by insect mortality. The first result given by Monte-Carlo tests was (Table 2) that the distribution of larvae was very close to the distribution of damages, as in most of the cases the tests were significant for both counts at the same scales. The spatial distributions of lar-

TABLE 2. Spatial analysis of the pattern of larvae and damages of EVM in vineyard G1P (first moth generation in Preignac). Monte-Carlo tests of the hypotheses H_R, H_I, H_G: scales $k = r \times c$ at which ranks of the indices are significant (>195). NS = non significant for all k (I_D, index of dispersion, V_s, local variance between stocks on the row, V_r, local variance between rows, I_{Ms}, Moran's index between stocks on the row, I_{Mr}, Moran's index between rows, I_{M4}, Morans's index between rows and stocks, $r(x_i, y_i)$ correlation between counts and number of grapes, $cov(x_i, y_{i-1} + y_{i+1})$ covariance between counts and number of grapes on neighbors).

Hypothesis	Index	Larvae	Damages
H_R	I_D	all k	all k
	V_s, V_r	all k	all k
H_I	I_{Ms}	1×1 4×1 6×1	1×1
	I_{Mr}	NS	NS
	I_{M4}	1×1	NS
H_G	$r(x_i, y_i)$	all k	–
	$cov(x_i, y_{i-1} + y_{i+1})$	1×1	–

TABLE 3. Spatial analysis of the pattern of larvae and damages of EVM in vineyard G2P (second moth generation in Preignac). Monte-Carlo tests of the hypotheses H_R, H_I, H_G: scales $k = r \times c$ at which ranks of the indices are significant (>195). NS = non significant for all k (I_D, index of dispersion, V_s, local variance between stocks on the row, V_r, local variance between rows, I_{Ms}, Moran's index between stocks on the row, I_{Mr}, Moran's index between rows, I_{M4}, Morans's index between rows and stocks, $r(x_i, y_i)$ correlation between counts and number of grapes, $cov(x_i, y_{i-1} + y_{i+1})$ covariance between counts and number of grapes on neighbors).

Hypothesis	Index	Larvae	Damages
H_R	I_D	1×1 2×1 4×1 6×1 12×1 21×1	1×1 1×2 2×2 4×1 4×2 6×1 12×1 21×1 42×1
	V_s	1×1 1×2 2×1 4×1 4×2 6×1 12×1 21×1	1×1 1×2 2×1 4×1 4×2 6×1 6×2 12×1 21×1
	V_r	1×1 2×1 4×1 6×1 12×1	1×1 2×1 4×1 6×1 12×1 21×1
H_I	I_{Ms}	NS	1×1 2×1
	I_{Mr}	NS	NS
	I_{M4}	NS	2×1
H_G	$r(x_i, y_i)$	all k	–
	$cov(x_i, y_{i-1} + y_{i+1})$	2×1	–

vae and damages (Table 2) were not random: ranks of the statistics I_D, V_s and V_r were significant whatever the scale. The counts (larvae or damages) were aggregated (ranks >195). Aggregation does not mean that vine stocks were correlated or infested independently of each other, but that some units were not infested while some were highly infested. As H_R was rejected for all values of k, it was not possible to draw conclusions, on the basis of these results, about the heterogeneity between rows. Differences of density in both larvae and damage counts occurred between rows. The fourth row was significantly less infested ($\bar{x}$ = 1.15 larvae per stock) and less damaged ($\bar{x}$ = 2.25 damages per stock) than the others.

Results of the tests of H_I answered the question of the spatial dependence of the infestations. Adjacent vine stocks on a row were not infested independently of each other (larvae and damages): the rank of I_{Ms} was significant (>195) for $k = 1 \times 1$. It was also significant for the number of larvae per unit of size $k = 4 \times 1$ and $k = 6 \times 1$, but not for the damage counts. The rank of I_{Mr} was never significant for larvae or damage counts. This result showed that adjacent vine stocks or groups of vine stocks on different rows were infested independently of each other. For larvae counts, the results were close to damage counts.

Whatever the scale, the number of larvae per stock (or group of stocks) depended on grape availability of the stocks (or of the group of stocks) (Table 2), as the observed correlation between infestation and grape availability was greater than all correlations calculated under the hypothesis of independence. When considering grape availability on the nearest neighbors, it was interesting to see that stock infestation depended on the number of grapes of the neighboring stocks at the scale of the individual stock $k = 1 \times 1$ (Table 2).

Spatial pattern of larvae and damages in vineyard G2P

The infestation per vine stock for the second generation of the moth was less than for the first generation, as there was only 1.04 larvae per stock (49% of infested stocks) and 1.48 damages per vine stock (Table 1), and 56% of the vine stocks were damaged. Monte-Carlo tests (Table 3) showed that the infestations (larvae or damages) were generally not randomly distributed (hypothesis H_R rejected for several k) and aggregation was demonstrated for several scales (ranks of the criteria >195). The same results were observed when considering the damages. At large scales, for $k = r \times c$, with $r > 21$ and $c > 1$, the spatial pattern for larvae was not significantly different from a random distribution, and it meant that there was no significant variation of density among stock infestations and damages between the 3 rows. For the larvae counts, units were infested independently of each other whatever the size of the unit (rank of the criteria never significant for H_I). Results concerning the independence between damage counts were different from those obtained for larvae counts: damage counts on adjacent vine stocks on the same row were correlated (rank of I_{Ms} was >195 when $k = 1 \times 1$ and $k = 2 \times 1$).

As for the first moth generation, stock infestation depended on grape availability on the stock (H_G with $r(x_i, y_i)$ rejected for all k) (Table 3). The influence of grape availability on the neighbors was also shown at small scales ($k = 2 \times 1$) (Table 3).

Spatial pattern of larvae and damages in vineyard G1W

This vineyard was highly infested with 3.42 larvae and 5.68 damages per vine stock (Table 1). 15% of the vine stocks were not infested and there was a great heterogeneity between the infestation of individual vine stocks [I_D = 2.65 for the number of larvae per stock (Table 1)]. Some of them were not infested while some others contained 19 larvae. The results of Monte-Carlo procedures (Table 4) showed that for all scales, the infestations (dam-

TABLE 4. Spatial analysis of the pattern of larvae and damages of EVM in vineyard G1W (first moth generation in Wachenheim). Monte-Carlo tests of the hypotheses H_R, H_I, H_G: Scales $k = r \times c$ at which ranks of the indices are significant (>195). NS = non significant for all k (I_D, index of dispersion, V_s, local variance between stocks on the row, V_r, local variance between rows, I_{Ms}, Moran's index between stocks on the row, I_{Mr}, Moran's index between rows, I_{M4}, Morans's index between rows and stocks, $r(x_i, y_i)$ correlation between counts and number of grapes, $cov(x_i, y_{i-1} + y_{i+1})$ covariance between counts and number of grapes on neighbors).

Hypothesis	Index	Larvae	Damages
H_R	I_D	all k	all k
	V_s, V_r	all k	all k
H_I	I_{Ms}	all k	all k
	I_{Mr}	all k	all k
	I_{M4}	all k	all k
H_G	$r(x_i, y_i)$	1×1 2×1 5×1	–
	$cov(x_i, y_{i-1} + y_{i+1})$	NS	–

ages or larvae) were not randomly distributed (H_R was always rejected against aggregation). Neighboring stocks or groups of adjacent stocks on the row, or on different rows, were correlated at all scales (H_I was rejected for all k). In the same row, the infestation of a stock was correlated with the infestation of its neighbor with a global trend to decrease from the first to the last stock of the row. Between rows, the infestations of neighboring stocks were also correlated as the within-row decrease was observed in the same way in all rows. Such results revealed a gradient of infestations. There was no significant difference in the density of the infestations or damages between the four rows.

The hypothesis of independence between stock infestation and the number of grapes on the stock was rejected at some scales (Table 4), while there was no influence of the number of grapes of the neighboring stocks on stock infestation [rank of $cov(x_i, y_{i-1} + y_{i+1})$ never significant] (Table 4).

DISCUSSION AND CONCLUSION

Spatial patterns of *L. botrana* larvae and damages

Statistical analyses of the spatial data of *L. botrana* revealed different spatial patterns. In all cases, there was a high variability at the elementary scale (one vine stock) which demonstrated aggregation.

In Wachenheim, spatial patterns could not be interpreted from a biological or from a sampling point of view because the gradient phenomenon was predominant. A gradual infestation could be explained by environmental conditions, such as wind direction or strength during flight for selecting oviposition site, but this hypothesis can't be confirmed.

For the first moth generation in Preignac, spatial pattern was highly aggregative as Geier et al. (1953) also found for *L. botrana* and *E. ambiguella* and variations in density infestations, which could reach 40% from a row to the other, were observed. On the same row, the infestations of adjacent vine stocks were correlated. Correlations were also observed on the row at the scale of 12 adjacent stocks. The different rows were independently infested. Aggregation could be explained by moth oviposition behavior and especially site seeking. Besides, female mobility for oviposition is quite limited (30 m) after mating (Schmitz, 1992) and seems to be more frequent among stocks within the same row than between rows. This could explain why infestations were correlated at some scales within a row. Larval mobility could also explain spatial pattern, but the exploration distances are certainly restricted to an individual stock even if moving could be observed during a long period (till 24 h) for neonate larvae of the first moth generation before penetration in the bud (Marchal, 1912).

In the same vineyard, the spatial pattern of the second moth generation was less structured than that of the first generation, and can be assimilated at some scales to random distribution. This could be attributed to the small means (near 1 per stock) for which it is difficult to distinguish randomness and aggregation (Upton & Fingleton, 1988). Aggregation was essentially observed at the scale of the stock and there was no variation in the density of infestations and damages between rows. This scale of pattern can be related to moth behavior rather than to vine stock heterogeneity because the subplot was different from that of the first generation. The infestations of neighboring stocks were independent.

Using the interpretable results (Preignac), we can conclude that the first scale for spatial heterogeneity is the vine stock, because aggregation was always detected at that scale. Positive correlation occurred for vine stock infestations on the same row but never between different rows. A spatial pattern was observed at the scale of 12 adjacent stocks on the same row. We can not compare our results with those obtained by Geier et al. (1953) because their spatial data was not collected on a plot exhaustively sampled, the size of their plot was greater (100 stocks × 80 planting rows) and two species were sampled, *E. ambiguella* being largely predominant.

The spatial pattern of the damages was, in all the cases, very close to that of the larvae. The damages as they were defined corresponded to the consumption of grape flowers (first moth generation) or of the berries (second moth generation) by the larvae. The mobility of the larvae being surely restricted to the vine stock where they have been deposited (Marchal, 1912), this result was not surprising.

Spatial relationship between stock infestations and grape availability

In all cases, the number of larvae per stock was correlated with the number of grapes of the stock, stocks with a lot of grapes being more infested than stocks with fewer grapes. Of course when there were no grapes on a stock, no larva was found on the stock. However, this did not occur in Wachenheim and was rare in Preignac (7% of the stocks had no grapes). From a spatial point of view, our results, when considering the number of grapes of the nearest neighbors on the row of a stock, suggested that the micro-environment of an individual stock had an effect on the moth. With a given number of grapes, a stock was more infested when it was surrounded by stocks with a lot of grapes than when it was surrounded by stocks with fewer grapes. This relationship was suggested for EVM by Geier et al. (1953) and Fermaud (1990), and was demonstrated for some other grape moths (Clark & Dennehy, 1988), due to the amount of grape volatiles. In our conditions, it occurred at small scales of 1 or 2 stocks on each row.

Implications for sampling

Estimating larval population density is a current objective when sampling populations. Defining a sampling method for this objective requires accurate identification of the population to be sampled and a degree of precision which is to relate to the use of the estimate and to the ramifications of an incorrect estimate. These are not the purposes of this study, but consideration must be given to these points when evaluating and proposing sampling procedures. Then the sample unit and the procedure by which the sample may be selected are needed. The sample unit must lead to independent observations and to a reduced sampling cost. The results obtained in this study showed that redundant information could be obtained when sampling adjacent stocks on the same row because infestations of adjacent stocks were correlated. This was unlikely because for a same required sample size, sampling adjacent stocks reduced surveyor movement in the vineyard and then sampling time. On the other hand, we have shown that spatial heterogeneity was maximum at the scale of the stock. For these two reasons, an individual stock seems to be a good sample unit.

On the basis of our study we can not propose a procedure by which the sample may be selected. It consists in defining the sample size and the plan to choose the units. The calculation of the sample size is based on specific studies to determine a model describing variability between sample counts, for example a variance-mean relationship. This can be done on the basis of a great number of surveys in different locations and years. Planning how to choose the sample requires also specific studies, because sampling plans must be simulated to calculate their sampling characteristics and then to test and to compare their efficiency. However, we have shown that at the scale of a vineyard, spatial heterogeneity could occur. Infestations could be different between rows, so that sampling along a row could lead to a non representative sample of the vineyard population. Therefore, if the population to be sampled is the vineyard population, sampling cannot be limited to units observed along a row.

Contribution of Monte-Carlo tests to detect spatial patterns

The statistical method that we have used allowed us to describe different spatial patterns and provided valid results as it needed no approximation about the distributional function of the indices. It may be largely adapted to other situations for ecological studies (Badenhausser, 1994; Vaillant & Hawlitzky, 1990). The conditions which are needed are to precisely identify the hypothesis tested (even a complex one), to choose indices and to simulate the hypothesis. For example, the spatial relationship between the density of grapes and the infestations, according to some models, can be studied by using these methods. More precisely, we can test if the number of larvae in unit i is a defined function, for example a positive proportionality, of the number of grapes of the same unit, or of the adjacent units. The simulations may consist in the redistribution of the larvae in the units according to this function, and covariance may be used as an index to test the hypothesis.

REFERENCES

BADENHAUSSER I. 1993: *Etude de la répartition du puceron Brachycaudus helichrysi Kalt. à l'échelle de la parcelle et de la plante de tournesol Helianthus annuus L. Apports pour l'échantillonnage.* Thèse, Université de Rennes, 109 pp.

BADENHAUSSER I. 1994: Spatial patterns of alate and apterous morphs of the Brachycaudus helichrysi (Homoptera: Aphididae) in sunflower fields. *Envir. Entomol.* **23**: 1381–1390.

BARNARD G.A. 1963: Discussion of the paper by Prof. M.S. Bartlett. *J. Stat. Soc. (Ser. B)* **25**: 294.

BESAG J. & DIGGLE P.J. 1977: Simple Monte Carlo tests for spatial pattern. *Appl. Stat.* **26**: 327–333.

CLARK L.G. & DENNEHY T.J. 1988: Oviposition behaviour of grape berry moth. *Entomol. Exp. Appl.* **47**: 223–230.

CLIFF A.D. & ORD J.K. 1973: *Spatial Autocorrelation.* Pion, London, 178 pp.

FERMAUD M. 1990: *Incidences des attaques larvaires d'Eudémis (Lobesia botrana) sur le développement de la pourriture (Botrytis cinerea) chez la vigne: rôle des facteurs du milieu et mécanismes mis en jeu.* Thèse de doctorat de l'INAPG, INRA, Paris, 104 pp.

GEIER P., MATHYS G., MURBACH R. & WEBER A. 1953: Estimation des populations de vers de la grappe (Clysia ambiguella Hb. et Polychrosis botrana Schiff., Lép. Tortric.). *Annu. Agric. Suisse* **2**: 953–958.

GREIG-SMITH P. 1952: The use of random and contiguous quadrats in the study of the structure of plant communities. *Ann. Bot. (N.S.)* **16**: 293–316.

HOPE A.C.A. 1968: A simplified Monte Carlo significance test procedure. *J. Stat. Soc. (Ser. B)* **30**: 582–598.

JOHNSON N.L. & KOTZ S. 1969: *Discrete Distributions.* John Wiley & Sons, New York, 328 pp.

LLOYD M. 1967: Mean crowding. *J. Anim. Ecol.* **36**: 1–30.

MARCHAL P. 1912: *Mission d'étude de la Cochylis et de l'Eudémis.* Librairie Polytechnique, Béranger, Paris, 326 pp.

PIELOU E.C. 1969: *An Introduction to Mathematical Ecology.* John Wiley & Sons, New York, 266 pp.

ROEHRICH R. & BOLLER E. 1991: Tortricids in vineyards. In Van Der Geest L.P.S. & Evenhuis H.H. (eds): *World Crop Pests 5: Tortricids Pests, Their Biology, Natural Enemies and Control.* Elsevier, Amsterdam, pp. 507–514.

ROEHRICH R. & SCHMID A. 1979: Lutte intégrée en viticulture. Tordeuses de la grappe: évaluation du risque, détermination des périodes d'intervention et recherche de méthodes de lutte biologique. In: *Proc. Int. Symp. IOBC/WPRS on Integrated Plant Protection in Agriculture and Forestry, 8–12 October 1979, Vienna, Austria.* IOBC/WPRS, France, pp. 245–254.

SCHMITZ V. 1992: *Contribution à l'étude du mécanisme de la confusion sexuelle des mâles chez l'insecte, application à la mise au point du procédé biotechnique de lutte contre l'Eudémis de la vigne Lobesia botrana Den. et Schiff.* Thèse de doctorat de l'ENSAR, INRA, Rennes, 113 pp.

TAYLOR L.R. 1961: Aggregation, variance and the mean. *Nature (London)* **4**: 732–735.

UPTON G.J.C. & FINGLETON B. 1988: *Spatial Data Analysis by Example. Vol. 1.* John Wiley & Sons, New York, 409 pp.

VAILLANT J. & HAWLITZKY N. 1990: Statistical analysis of occupancy rates for overdispersed populations by redistribution procedures: application to the European corn borer egg masses distribution. *Res. Popul. Ecol.* **32**: 289–301.

VENABLES W.N. & RIPLEY B.D. 1994: *Modern Applied Statistics With Splus.* Springer, New York, 462 pp.

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