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Evaluating the spatial distribution of *Dociostaurus maroccanus* egg pods using different sampling designs

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Abstract

In its gregarious phase the locust *Dociostaurus maroccanus* (Thunberg) (Orthoptera Acrididae) has periodically caused significant yield losses in many Mediterranean and Asian countries, and alarm in the general public. Population outbreaks in recent years have frequently required the application of control measures, based on those that have low environmental impact, which are only possible with a sound knowledge of locust bio-ethology and ecology. Our research was aimed at studying the spatial distribution of *D. maroccanus* egg pods in two Apulian egg bed areas in southern Italy, thus contributing to the rationalization of control methods. The distribution of *D. maroccanus* egg pods was investigated using a geostatistical approach. Three sampling designs (called A, B and C), characterized by different mesh and clod sizes, were compared to evaluate their effectiveness and affordability. In both egg bed areas, the variogram models were asymptotic with a small nugget effect, and indicated an aggregated distribution of egg pods. Contour maps showed that design A, based on a larger mesh and clod size, was characterized by few hot spots and an extended zone of "low density" egg pods, while design B, involving a smaller mesh and clod size, showed a more structured distribution, with various hot spots alternating with zero level zones. Finally, design C, based on a larger mesh size and smaller clods, showed a single extended hot spot surrounded by a large area without egg pods. Moreover, because of the larger amount of soil to be examined, design A was about 2.6- and 10.9-fold more time consuming than designs B and C, respectively. Our data showed that sampling designs providing smaller and denser samples should be preferred over designs with fewer and larger samples when information on both the distribution and density of egg pods is needed.

Key words: Orthoptera, Acrididae, Apulia, geostatistic, oothecae distribution, bio-ethology.

Introduction

Dociostaurus maroccanus (Thunberg) (Orthoptera Acrididae) is a univoltine species that is active from April (as nymphs) to August and spends the rest of the year as eggs laid in oothecae, normally called egg pods, in the upper layers of the soil.

In its gregarious phase, voracious swarms of this grasshopper have often occurred in regions of southern Italy (Targioni Tozzetti, 1882; Melis, 1933; Jannone, 1934; Paoli, 1937; Moleas *et al.*, 2002), other Mediterranean countries, Asia Minor, and the Middle East to Afghanistan and south-east Kazakhstan (Uvarov, 1932; 1933; Moreno Márquez, 1945; Delmas and Rambier, 1951; Latchininsky and Launois-Luong, 1992; Latchininsky *et al.*, 2002), causing serious yield losses to cereals and other crops.

The environmental sustainability of a strategy aimed at controlling locusts is based on the use of active insecticide substances with a low toxicity to non target organisms, on the timeliness of treatments and on restriction of treatment to the smallest possible area. Significant progress has been made in the search for locusticides with low environmental impact, spanning from organochlorine compounds to spinosyns, and on the use of entomopathogenic fungi such as *Metarhizium anisopliae* (Metschnikoff) (Baldacchino *et al.*, 2004).

Knowledge of the precise localization of *D. maroccanus* hatching nymphs is required in order to perform timely treatment of the smallest possible area. The identification of these areas, the so-called egg beds, can be facilitated by following swarms of adult females to oviposition sites. However, the availability of reports of breeding areas is not sufficient to trigger specific treatment. The need for a spring treatment should take into account: i) the extent of the egg beds (small isolated egg beds are not necessarily a threat to adjacent crops); ii) the density of vital eggs (taking into account that egg pods are subject to predation by some Diptera Bombyliidae and Coleoptera Meloidae); iii) the trend in egg hatching (hatching is not uniform within a single egg bed, being influenced by soil temperature).

Knowledge of the spatial distribution of egg pods is a prerequisite for an efficient and economically sustainable strategy for assessing such factors. Visual monitoring during the oviposition period can facilitate the location of the egg bed areas, but only soil sampling can provide data on egg pod density, their rate of predation and hatching.

The introduction of geostatistics in pest monitoring opened new possibilities for the study and management of the spatial patterns of insects at different stages. Geostatistics provides a powerful tool with which to model, estimate and represent spatially explicit correlated data (Liebhold *et al.*, 1993). Applications in applied entomology are various and include the study of the spatial distribution and dispersion of populations at various scales, the identification of pest infestations in agroecosystems and the incorporation of spatial information into management plans (Brenner *et al.*, 1998; Fleischer *et al.*, 1999; Sciarretta *et al.*, 2001; 2008; Nestel *et al.*, 2004; Park *et al.*, 2007; Albieri *et al.*, 2010; De Luigi *et al.*, 2011; Burgio *et al.*, 2012).

Geostatistical studies on Orthoptera are rather limited and mainly focused on the adult stage. Johnson and Worobec (1988) and Kemp *et al.* (1989) produced maps of grasshopper abundance on an area-wide basis; Johnson (1989) analysed the spatial autocorrelation of grasshoppers in roadside survey counts to predict population densities in crop fields; locust spatial densities were correlated with host plants along the coastal plain in Sudan and distributional maps were obtained (Woldewahid *et al.*, 2004).

The aim of our study was to investigate the distribution of *D. maroccanus* egg pods in two egg bed areas in southern Italy, using different sampling designs. The obtained spatial patterns were compared to assess their spatial resolution and the affordability of sampling designs in terms of labour.

Materials and methods

Study area

The study was carried out in pastures located near the town of Manfredonia in Apulia, Southern Italy. For the purposes of this study only data obtained from egg beds in pastures close to the localities Posta del Fosso (41°38'25.17"N, 15°52'51.43"E, at 70 m a.s.l.) and Posta Garzia (41°38'6.73"N, 15°52'55.61"E, at 57 m a.s.l.) were used in the geostatistical analysis, as those sites were the largest and richest in egg pods. The pastures were part of a wider garrigue phytoclimatic association, characterized by an abundance of outcropping rock and the presence of daffodils, thistles, squills, thyme and small trees or shrubs of wild pear, olive and terebinth. Fields surrounding the pastures were planted with wheat, barley, oats and forage crops, interspersed with olive and almond groves (Moleas *et al.*, 2002).

By following the ovipositing *D. maroccanus* females in June and July, egg beds were localized and appropriately marked for sampling in subsequent seasons.

Field data collection and sampling procedure

During the winter-spring period, samples of soil up to 10 cm in depth were taken from the egg beds in accordance with the following sampling designs (figure 1):

- Sampling design A - Samples of soil of 50 × 50 cm in a grid with a mesh of 20 × 20 m;
- Sampling design B - Samples of soil of 15 × 15 cm in a grid with a mesh of 10 × 10 m;
- Sampling design C - Samples of soil of 15 × 15 cm in a grid with a mesh of 20 × 20 m.

The third design (C) was a simulated sampling strategy, achieved by considering only the samples of soil in design B which were in the same position as in A. In this way, the resulting design was the same as that in A, but with sample sizes of 15 × 15 cm.

General information on the designs, including the dimensions of the sampled clods and the distances between sampling points, is given in table 1.

Samples of 50 × 50 cm were transported to the laboratory and examined for egg pods, while the clods of 15 × 15 cm were examined directly in the field.

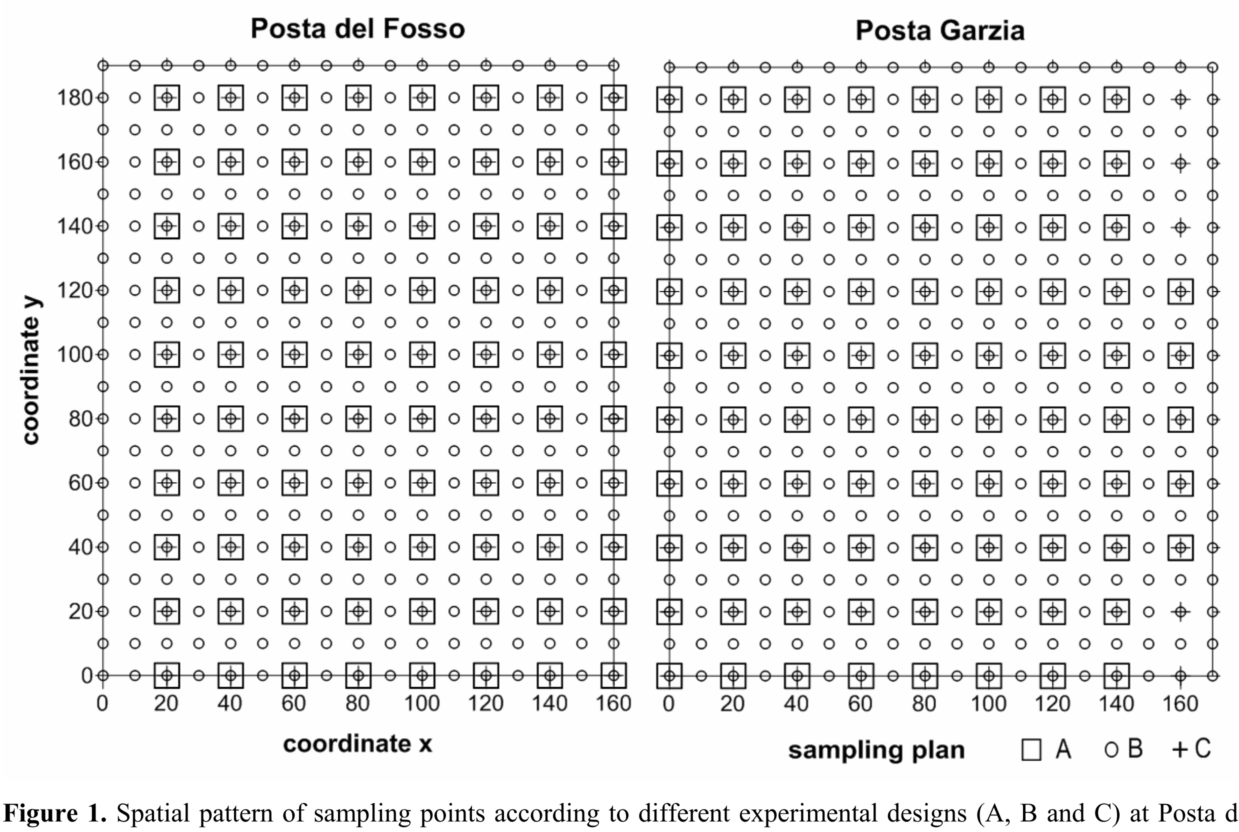

Figure 1. Spatial pattern of sampling points according to different experimental designs (A, B and C) at Posta del Fosso and Posta Garzia.

Table 1. General characteristics of the sampling designs with clods of 50 × 50 cm (design A) and 15 × 15 cm (design B and C) at the sites Posta del Fosso and Posta Garzia.

	Posta del Fosso			Posta Garzia		
	Plan A	Plan B	Plan C	Plan A	Plan B	Plan C
Surface area of laying sites (ha)	2.9	3	2.9	2.9	3.2	2.9
Number of samples	80	340	80	85	360	85
Distance between samples (m)	20 × 20	10 × 10	20 × 20	20 × 20	10 × 10	20 × 20
Maximum distance (m)	240	250	240	240	255	240
Surface area of single sample (m^2)	0.25	0.02	0.02	0.25	0.02	0.02
Total surface area sampled (m^2)	20	7.65	1.8	21.25	8.1	1.9
Total volume sampled (m^3)	2	0.76	0.18	2.12	0.81	0.19
Surface area sampled (%)	0.070	0.025	0.006	0.070	0.025	0.006

Given the different grid and clod sizes used in the experimental design, comparisons between designs and between sites were conducted using elementary statistics based on the density of egg pods per m^2 of surface (table 2).

During the analyses, the time spent working on each sample was noted to evaluate the suitability of the operation.

Identification

Several hundred oothecae were kept in the laboratory until the eggs hatch and the larvae were reared to obtain adults. All nymphs and adults obtained proved to belong to *D. maroccanus*.

Spatial analysis

Geostatistical methods were used to characterize the spatial distribution of *D. maroccanus* egg pods in the three experimental sampling designs (A, B and C), expressed as density (number of egg pods per m^2). By comparing sampling designs B and C, we evaluated the effect of grid density on spatial distribution without the effect of sample size.

The spatial dependence among observations was examined by calculating omnidirectional semivariograms with a maximum distance of 120 m. The variogram analysis was performed using GS+ Version 7 (Gamma Design Software, Plainwell, Michigan, USA). Since a proportional effect was observed for all data sets, the data were transformed to log (x+1) prior to analysis (Journel and Huijbregts, 1978). Experimental variograms were fitted according to the model that gave the lowest residual sums of squares (RSS). Linear, spherical, exponential and gaussian functions were tested by the software. Models were defined by the nugget (C_0), the range (a) and the sill (C). The ratio C_0/C, known as the *k* parameter, was used to evaluate the amount of randomness that exists in the data at distances smaller than the sampling distance. Values below 0.8 indicate that the distribution is aggregated, with a strong relation between samples (Journel and Huijbregts, 1978). The range allows an estimate to be made of the distance at which two points are no longer correlated and, thus, the minimum distance between sampling points that are statistically and spatially independent (Bacca *et al.*, 2006; Park and Tollefson, 2006).

Models obtained from variogram analyses were used to interpolate *D. maroccanus* egg pods by means of the kriging algorithm. Spatial analyses were carried out using Surfer software Version 9.5 (Golden software, Golden, Colorado, USA) with *x*, *y* representing the spatial coordinates and *z* the egg pod density. The resulting interpolation grid was graphically represented by a contour map, which shows the configuration of the surface by means of isolines representing equal z-values; a base map showing the experimental area, with the same coordinate system, was placed on top of the contour map. Zones of the contour map with higher egg pod density than surrounding areas are referred to as "hot spots".

Kriging interpolation was evaluated by cross-validation analysis, in which each measured point was individually removed and its value estimated in the absence of the specified value, to determine the quality of model fitting. The mean predicted error, the rooted mean squared error (RMSE) and the mean squared deviation ratio (MSDR) were obtained from residuals, which were calculated by comparing estimated *vs.* actual values (Webster and Oliver, 2001). Calculation of RMSE and

Table 2. *D. maroccanus* egg pods collected at the two sites using various sampling designs.

	Posta del Fosso			Posta Garzia		
	Plan A	Plan B	Plan C	Plan A	Plan B	Plan C
Total egg pods (N)	2786	1438	214	1124	524	117
Mean density per sample	34.83	4.22	2.38	13.22	1.46	1.30
SE density per sample	7.13	0.62	0.53	3.33	0.32	0.30
Maximum density per sample	383	110	27	162	96	14
Mean density per surface unit (N/m^2)	139.3	188.0	118.9	52.9	64.7	57.8
SE density per surface unit	28.5	27.8	26.4	13.3	14.4	13.4
Zero samples (%)	23	60	63	53	76	72

MSDR enables an evaluation of the ability of the model to interpolate the experimental semivariograms: the lowest is RMSE, the highest is the accuracy of the model. Similarly, if the model for the variogram is accurate, then the mean squared error would tend to be equal to the kriging variance and MSDR would approximate to 1 (Webster and Oliver, 2001).

To validate the interpolation method, the maps of the actual values were compared with the maps of the estimated values, built using the same variogram models as before.

Results

Density of egg pods

A total of 2786, 1438 and 214 egg pods were collected at Posta del Fosso and 1124, 524 and 117 at Posta Garzia, following sampling designs A, B and C, respectively.

At Posta del Fosso the lowest egg pod density per surface unit was found in the sampling design C, whereas the highest was found in B and intermediate values in A. The standard error ranged between 26.4 and 28.5. In design A, 23% of samples was found without egg pods; in B the percentage of zero egg pod samples was significantly higher (60%), while in C it was similar to B (63%).

A lower density of egg pods per surface unit was generally found at Posta Garzia compared to Posta del Fosso, but in contrast to the latter, the lowest egg pod density was observed in design A, the highest in B and an intermediate value in C. The standard error of the mean density calculated at Posta Garzia was lower than at Posta del Fosso, ranging between 13.3 and 14.4. The percentage of zero samples in all three sampling designs at Posta Garzia was higher than at the other site (table 2).

Spatial distribution of egg pods

At Posta del Fosso, the variogram models were asymptotic (figure 2a), with a small nugget effect and the *k* parameter varying between 0.06 and 0.13, all of which indicate an aggregated distribution (table 3). The estimated range of models varied from 20.4 to 46.8 m (table 3).

Contour maps obtained from experimental data highlighted different spatial patterns (figure 3a): design A showed few hot spots and an extended area of "low density" egg pods; in B, a more structured distribution, with various hot spots alternating with zones without egg pods, was observed; in C, a single extended hot spot was surrounded by a large area without egg pods.

Cross validation results indicated notably low mean errors of the estimate (table 3). Design A produced the model with the lowest RMSE, whereas design B had the best MSDR value (table 3). Comparison of contour maps

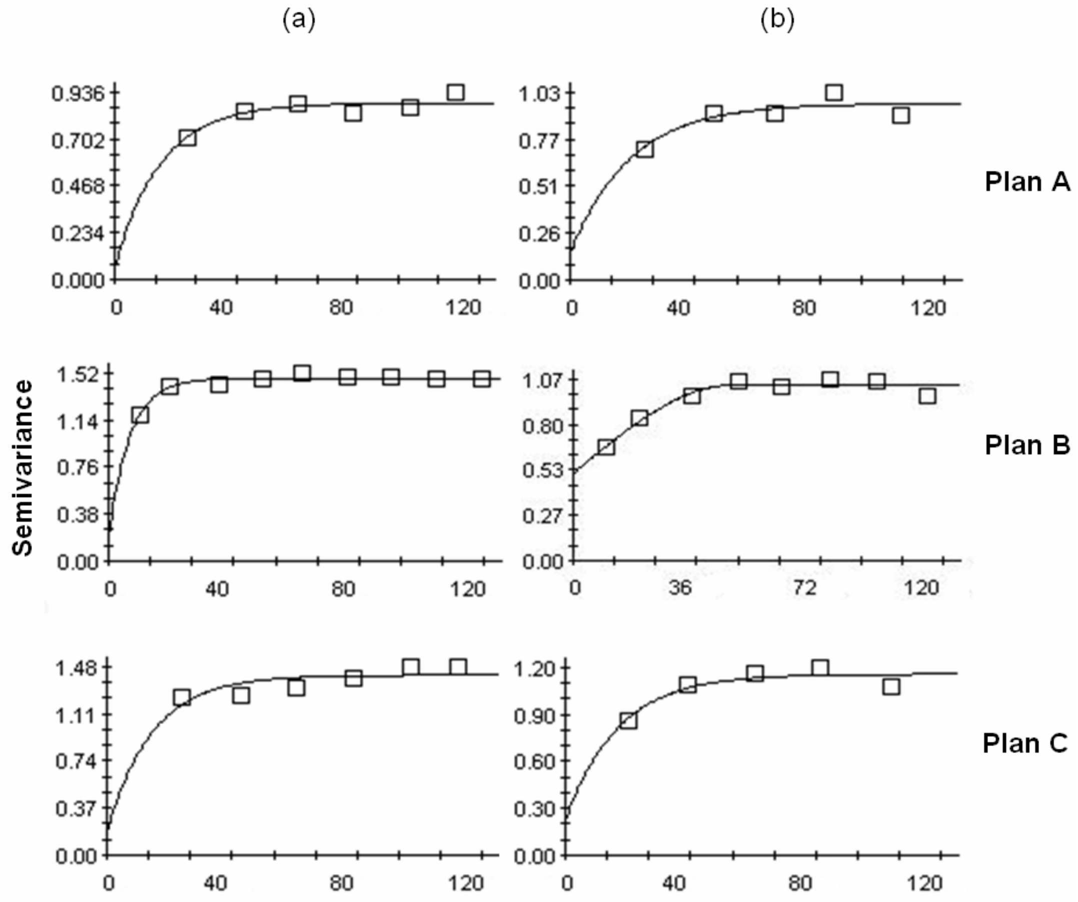

Figure 2. Experimental variograms obtained for designs A, B and C at Posta del Fosso (a) and Posta Garzia (b).

Table 3. Models and parameters obtained by fitting the semivariogram of the *D. maroccanus* egg pod density and results of the cross validation analysis carried out for the different sampling designs at the two sites Posta del Fosso and Posta Garzia.

	Posta del Fosso			Posta Garzia		
	Plan A	Plan B	Plan C	Plan A	Plan B	Plan C
Max distance	120	120	120	120	120	120
Lag interval	17	14	17	20	14	23
Number of lags	6	9	6	5	9	5
Model	Exponential	Exponential	Exponential	Exponential	Spherical	Exponential
RSS[a]	0.004	0.004	0.027	0.009	0.008	0.009
Nugget	0.04	0.18	0.18	0.14	0.51	0.22
Sill	0.94	1.47	1.42	0.96	1.03	1.16
Range (m)	46.8	20.4	42.9	57.3	46.9	49.8
k	0.06	0.13	0.13	0.15	0.50	0.19
Mean estimated Z	1.40	0.93	0.94	0.75	0.51	0.61
Mean error	0.005	0.004	0.005	0.004	0.001	0.003
RMSE[b]	0.964	1.156	1.19	0.83	0.86	0.97
MSDR[c]	0.987	0.998	0.987	0.994	0.999	0.994

[a] RSS: residual sums of squares; [b] RMSE= rooted mean squared error; [c] MSDR= mean squared deviation ratio.

based on the estimated density of the various sampling designs with those generated using the actual data highlighted a different situation (figure 3b): design B showed a similar pattern of distribution; A showed a loss of information, with the disappearance of hot spots observed in the original data; C showed few changes in the distribution.

At Posta Garzia, the variogram models were asymptotic (figure 2b), with design B showing a higher *k* parameter than A and C, but still indicating an aggregated distribution (table 3). The estimated range of models varied from 46.9 to 57.3 m (table 3).

Contour maps of the experimental data are shown in figure 4a. In design A, areas with equal contour levels extended over the sampled area, while in design B the hot spots contained higher densities of egg pods and occupied a limited part of the egg bed. In the case of design C, a single hot spot was located in the middle of the sampled area.

As for the other site, cross validation results highlighted low mean errors of the estimate (table 3). The lowest RMSE was calculated for design A, and the best MSDR was obtained for design B. Maps of the estimated density were similar to those obtained from the actual data in the case of designs B and C; an underestimation of the egg pod density was observed in design A (figure 4b).

Sampling affordability

The length of time required to crumble a clod of 15 × 15 cm, and then find and count all the egg pods laid in the soil was about 11 minutes, compared with the 2 hours required to inspect a clod of 50 × 50 cm.

In our experiments the largest mesh was 20 × 20 m. Only one clod of 50 × 50 cm was included in this mesh in sampling design A, with one of 15 × 15 cm in design C, and four clods of 15 × 15 cm in design B. Thus, in order to examine one clod representative of the same portion of soil (a single mesh), 2 hours were required for design A, 44 minutes for B, and only 11 minutes for C. As a consequence, about 160 hours were spent examining all soil samples from Posta del Fosso, which followed design A, and at which a total surface area of 20 m^2 and a total volume of 2 m^3 of soil were examined, about 59 hours were required for design B, with a total surface area of 7.65 m^2 and a total volume of 0.76 m^3, and about 15 hours were required for design C, with a total surface area of 1.8 m^2 and a total volume of 0.18 m^3 (table 1).

Discussion

The geostatistical analysis highlighted an aggregated distribution of *D. maroccanus* egg pods at both sites, as indicated by values of the *k* parameter. The estimated range of variograms can be used to calculate the aggregation area. With the exception of design B at Posta del Fosso, where the range was half that of the other designs, all values were comparable and ranged between 46.8 and 57.3 m, suggesting that the extent of the aggregation areas is not strongly influenced by the size of the sampling mesh.

The difference observed between variogram models calculated for design A and B at Posta del Fosso suggests that an aggregation area may have a more structured pattern at a finer resolution.

In fact contour maps of design B (i.e. with highest density of sampling points) showed a more detailed spatial pattern at both sites compared to designs A and C, indicating an effect of the grid mesh on the spatial resolution of the interpolated data.

In Extremadura (Spain), Arias *et al.* (1994) found that the densities of *D. maroccanus* egg pods decreased from the centre to the periphery of each of the four investigated sites, forming aggregation isles that, given the sampling scale used by those authors, covered a similar area to the hot spots found at the Italian sites in this study.

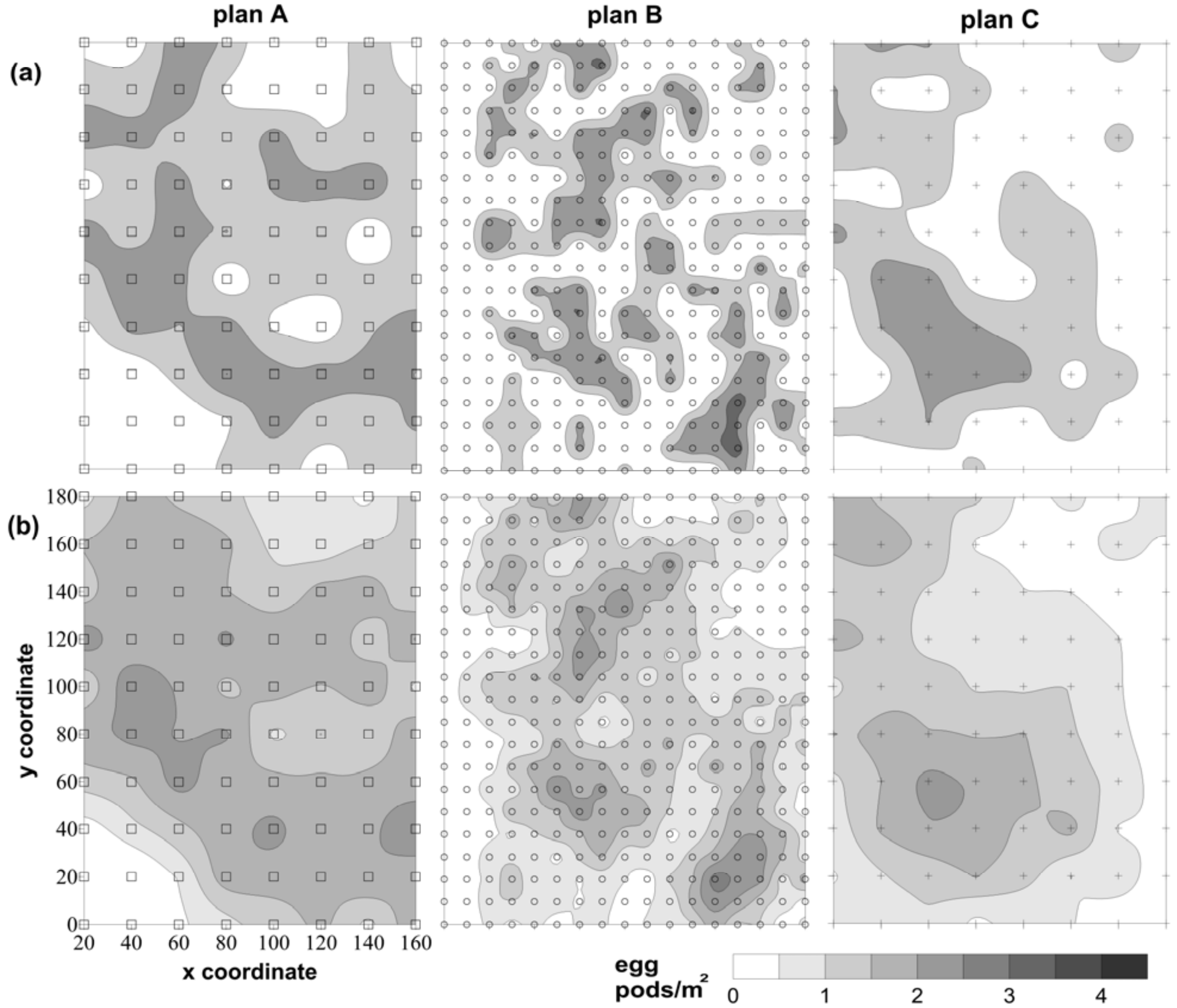

Figure 3. Distributional maps of *D. maroccanus* egg pods obtained from experimental (a) and estimated (b) data, using the variogram models of different sampling designs used at Posta del Fosso.

Due to the lack of other studies on the spatial distribution of Acrididae egg pods, only a few comparisons can be made with papers dealing with locust oviposition; however, an aggregated distribution has been widely observed.

In a very detailed study carried out in Cyprus, Merton (1959) took into consideration also the oviposition ecology of *D. maroccanus*. He studied the overall density and distribution of egg pods on several sites by means of a lattice sampling technique, using one-foot and two-inche wire squares. However, the author did not report any consideration on the effectiveness and affordability of the two types of sampling squares because his main aim was to show the distribution of egg pods in relation to topography and vegetation also determining the nature of the ground chosen for oviposition. Merton (1959) confirmed that soil structure was of great importance in determining the distribution of egg pods within the bare ground available for oviposition. In southern Nigeria, the aggregation of egg pods of the grasshopper *Zonocerus variegatus* (L.) seemed to be due to the preference of ovipositing females for soils that were permanently shaded under woody plants or soils close to cassava plants (Page and McCaffery, 1979). Using the Taylor's power law Shah *et al.* (2000) reported that the egg pods of the Acrididae *Hieroglyphus daganensis* Krauss and *Cataloipus fuscoceruleipes* Sjostedt occurring in northern Benin were highly aggregated under shrubs of *Piliostigma thonningi* (Schumach.) Milne-Redhead and *Vetiveria nigritana* (L.) for both grasshopper species, but regularly distributed under *Sorghum bicolor* (L.) Moench for *C. fuscoceruleipes*. These two studies show that in arid environments with high insolation the females of some species of Acrididae prefer to oviposit in soils protected by the shade of trees or shrubs rather than in sunny pastures, as occurs for *D. maroccanus* in Mediterranean countries. In Foggia's flat land, Paoli (1937) found *D. maroccanus* females converging and ovipositing in small areas of about 1 to 2 metres in diameter, separated from each other by small clumps or strips of lush vegetation, unevenly distributed over several square kilometres of hard and bare soils. Paoli's observations are consistent with our data, which suggest that at Posta del Fosso and Posta Garzia *D. maroccanus*

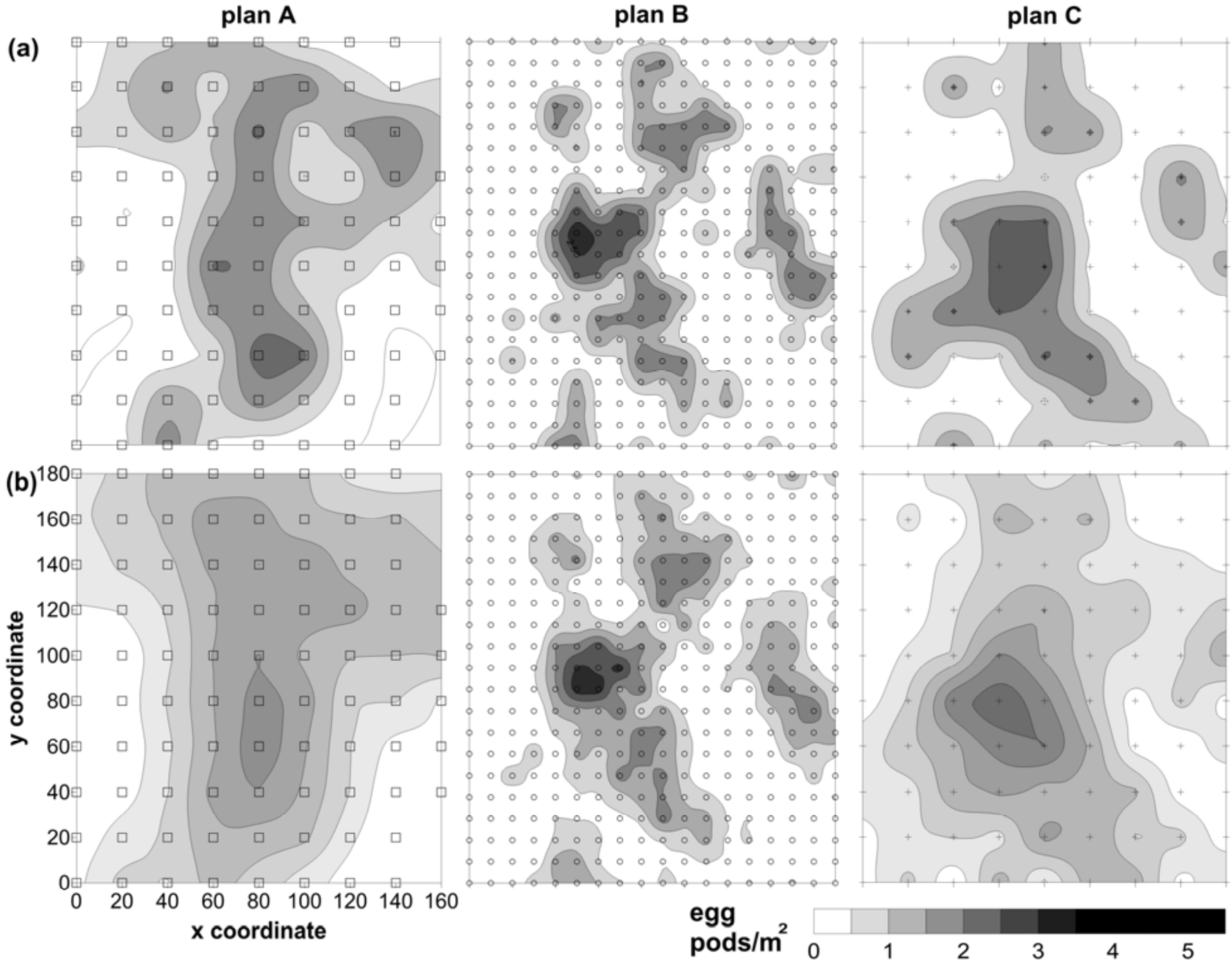

Figure 4. Distributional maps of *D. maroccanus* egg pods obtained from experimental (a) and estimated (b) data, using the variogram models of different sampling designs used at Posta Garzia.

females oviposited in an aggregated pattern; however, no clear differences regarding vegetation and soil were found to justify the preferences shown by females for ovipositing in a particular spot.

At Posta del Fosso, sampling design A was about 2.6- and 10.9-fold more time consuming than designs B and C, respectively. Due to the larger number of samples, the manpower required to examine all samples collected at Posta Garzia was slightly higher than at Posta del Fosso. However, design A was again about 2.6- and 10.9-fold more time consuming than designs B and C, respectively.

Despite the greater amount of time required to examine samples in design A, the experimental data suggest that there was no improvement in accuracy when estimating the mean density of egg pods per square metre of surface. In fact, the standard error of density per surface unit was fairly similar in the three designs within each site (table 2). Furthermore, distributional maps of *D. maroccanus* egg pods obtained from experimental design A were less informative compared to B (figures 3 and 4), providing a more flattened distribution and masking the presence of numerous hot spots. Thus we can conclude that, in order to obtain information on both the distribution and density of egg pods, sampling designs providing small (15 × 15 cm) and more dense samples (10 × 10 m) should be preferred to designs with larger (50 × 50 cm) and fewer samples (20 × 20 m).

Although the least time consuming sampling design was C, it did not guarantee the same accuracy in describing both the distribution and density of egg pods as design B. This was verified at both Posta del Fosso and Posta Garzia. For example, inspection of the distributional map of egg pods shows that the contour map of design C was more similar to that of A, but at the same time it showed a loss of information compared to B (figures 3 and 4).

On the contrary, some of the descriptive statistics (like mean density per surface unit, standard error of density and percentage of zero samples) for design C were more similar to B than to A. Thus sampling plans such as C, characterized by a small number of samples and a relatively large mesh, can be used if the accuracy of the estimate is not particularly important, and may be appropriate for sampling presence/absence. Badenhausser *et al.* (2007) estimated Acridid adult densities in grassland habitats using a different approach, and showed that, for a fixed precision of > 0.35, presence/absence sampling required less time than enumerative sampling when densities were low (< $2/m^2$). This implies that a large reduction in sample numbers (as in design C) can affect the level of precision of the survey, especially at higher densities.

Compared to the method proposed by Lecoq and Mestre (1988), which suggests randomly collecting at least 10 squares of soil each with a surface of 1 m^2 to estimate the egg pod density of a site, design B and particularly design C of the present work are clearly less time consuming, at the same time providing a good estimate of egg pod density.

Each sampling plan has its advantages and disadvantages. When the priority is economic sustainability, as may be the case when monitoring locusts on a large scale, designs involving fewer and smaller samples (like design C) may be preferred since they require the smallest volume of soil, thus being the least time consuming. Moreover, small samples of soil (required by designs B and C) can be examined directly in the field, avoiding problems linked with their transport. Therefore, the least time consuming sampling design represents the minimum effort required when planning rational control of the locust, based on the estimate of the density of viable eggs, the hatching of which will give the 1st instar larvae, the most vulnerable and aggregated stage of the insect. On the other hand, the egg pod distribution obtained using design C appears the least informative of the three compared here. Thus, when the main aim of the survey is the accurate characterization of the density and distribution of egg pods, sampling designs involving small but numerous samples of soil should be preferred since they lead to more precise contour maps and more easily identifiable hot spots. Although more expensive, this more detailed survey of egg pod distribution, allowing the prediction of areas with high densities of nymphs, could be useful when planning localized treatment of the 1st instar to minimize the side effects of insecticides on beneficial arthropods.

Overall, the sampling designs considered in this study may provide a powerful tool for enhancing the economic and environmental sustainability of the control of *D. maroccanus*, allowing the estimation of egg pod distribution and density in the egg beds identified in the summer.

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