Estimating Acridid Densities in Grassland Habitats: A Comparison Between Presence–Absence and Abundance Sampling Designs

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Environ. Entomol. 36(6): 1494–1503 (2007)

ABSTRACT Sampling methods to estimate acridid density per surface area unit in grassland habitats were compared using presence–absence data and count data. Sampling plans based on 6 yr of surveys were devised to estimate the density of *Chorthippus* spp., *Euchorthippus* spp., and *Calliptamus italicus* L. These acridids represented >90% of species in the study area. Sampling plans based on count data provided a reasonable tool when densities were $>1/m^2$ and when the level of precision was 0.20–0.30. A binomial sampling plan can be used to estimate *C. italicus* density with a level of precision ≥0.28. Sampling characteristics, i.e., estimated mean, actual precision, and sample size, were established on validation data sets with bootstrapping analysis. Sampling costs were also calculated according to density-dependent functions. Comparison between binomial sampling and enumerative sampling of *C. italicus* showed that binomial sampling required less time than enumerative sampling when densities were $\leq 2/m^2$ and when fixed precision was >0.35. Plot area had no significant effect on sample variances of counts.

KEY WORDS sampling, grasshopper, Italian locust, *Calliptamus italicus* L., Gomphocerinae

Acridids (grasshoppers and locusts) are among the major insect inhabitants of grasslands and are a dominant component of biodiversity in terrestrial ecosystems (Baldi and Kisbenedek 1999). In addition to the direct effect of reducing standing crop, they can influence ecosystem processes by increasing nutrient leaching from foliage, defaecation, changing plant resource allocation, and plant community structure. However, they are major preys for other invertebrates and for predatory vertebrates, notably snakes and birds. Moreover, acridids are considered indicators of agricultural change and its environmental consequences (Wingerden et al. 1992). Estimates of acridid population parameters are therefore essential in ecological studies and in management strategies.

Acridids are inherently difficult to sample because of their jumping and flying behavior (Browde et al. 1992). Studies of acridids use a wide range of sampling techniques and designs. Onsager (1977) showed the most reliable methods for estimating acridid abundance were those that physically delineate the boundaries of the sampling universe (e.g., cage sampler) compared with visual estimates and sweep nets, which are more commonly used (Bridle et al. 2001, Guido and Gianelle 2001). However, the sampling cost associated with these methods was high compared with sweep net and visual estimates. In most of these studies for which sampling issue is to estimate acridid population parameters such as density per surface area, we lack an evaluation of sampling design, except for some pest species (Legg and Lockwood 1995, 2001).

Sampling designs in which the sample units are chosen at random are strongly recommended because they give a better estimate of population variance (Williams et al. 2002). Furthermore, the frequency distributions of the population parameters are usually unknown, and therefore, simple random sampling is the preferred design for estimating the population mean.

Thus, questions about choosing a sampling design will eventually result in questions about the sample size. To answer this question, an estimate of count variance is necessary to assess the precision of population estimates (Anscombe 1952). As a general rule in insect sampling, variance is correlated to the mean, and modeling the relationship between variance and mean allows for assessing the optimal sample size (Taylor 1961, Southwood 1978). Retrieving and counting all insects present in a sample unit can be tedious, and thus a sampling strategy based on the presence-absence might be a useful alternative for estimating population density more rapidly. Binomial sampling requires a statistical relationship between the proportion of occupied sample units and population density (Nyrop et al. 1989) and also requires that the proportion of occupied samples, P_1, is not saturated, i.e., $P_1 = 1$, too quickly. Sampling designs to estimate acridid densities have previously been compared in North America and Africa (Pfadt 1982, Browde et al. 1992, Schell and Lockwood 1997, Legg and Lockwood 2001, Woldewahid et al. 2004), but these studies had high densities of pest species. Furthermore, these species belong to different genera compared with dom-

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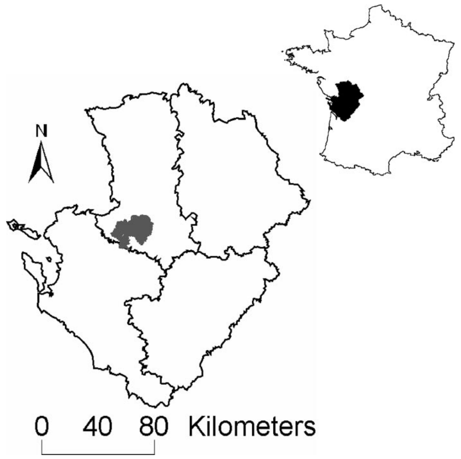

Fig. 1. Study area in Poitou-Charentes region in France.

inant species of European grasslands, i.e., *Chorthippus* spp., *Euchorthippus* spp., *Calliptamus italicus* L., and *Pezotettix giornai* Rossi (Baldi and Kisbenedek 1997, Badenhausser and Bretagnolle 2005, Reinhardt et al. 2005). Apart from *C. italicus*, the aforementioned list rarely reaches high densities and are not considered pests; as a result, sampling plans have not been created for most European grassland acridids.

The objectives of this study were to provide sampling designs based on random sampling to estimate acridid population densities in grassland habitats with a fixed level of precision. The sample unit consisted in a cage sampler as recommended by Onsager (1977). The first sampling design used acridid counts per cage sampler and was based on Taylor's power law (Taylor 1961). The second sampling design used presence-absence data, i.e., at least one acridid per cage sampler and was based on the model of Gerrard and Chiang (1970). Models were based on extensive sampling data over a 6-yr period. We compared the two sampling approaches for the two dominant acridid taxons encountered in our grassland habitats. We considered the accuracy of estimate mean and variance parameters by simulating sampling designs with bootstrap samples (Naranjo and Hutchison 1997), as well as the time cost of sampling calculated as a function of the sample size, and of the number of sample units containing k acridids.

Materials and Methods

Study Area. Data were collected in a 350-km^2 study area, located within the Poitou-Charentes Region (Département des Deux-Sèvres, 46.11° N, 0.28° W) in Western France (Fig. 1). This area is dominated with intensive agriculture, although it used to be a mixed-farming system. Recent agricultural intensification has reduced grasslands ~10% from ~70% in 1970. The study site consists of ≈12,000 parcels, mostly dedicated to cereal crop production. The study site was limited by the city of Niort on the northwest, the Chizé Forest in the south, and by valleys or hedged farmland (e.g., small fields with a dense network of hedgerows) in the north, east, and inside (Fig. 1). Since the start of the study in 1995, land use on every single agricultural field has been recorded annually in the field and mapped onto a Geographical Information System.

Field Data Collection and Grasshopper Sampling Procedure. Data were collected over a 6-yr period, from 1999 to 2004. Seventeen (2001) to 102 (2004) plots were randomly chosen among the grassland fields of the study area (Table 1). The different types of grassland crops included leguminous fields, such as alfalfa and clover, and herbaceous grasslands managed by grazing, mowing, or set aside (Table 1). Acridid populations in a field were sampled once per year, each year over a 15-d period from mid-July to mid-August. In 2003, six plots were added, and acridids were sampled on seven dates (every 15 d from June to October) to assess their population dynamics (Table 1). All data have been combined for this analysis.

Acridids were sampled on a field basis by means of removal-trapping with a 1-m^2 square cage sampler. The cage sampler was thrown 10 times at random within the field and constituted the sample unit. The

Table 1. Sampling period and no. and type of grassland field samples used for establishment of the variance–mean relationship and for Gerrard and Chiang relationship, average density (SE) of acridids per cage sampler (1 m^2), and sample distribution according to acridid density

Year	Sampling period	Number of plots							*C. italicus*		*Chorthippus*/*Euchorthippus*			All species
		Meadow	Pasture	Shrub	Set-aside	Alfalfa	Clover	Total	$N > 0^a$	Mean (SE)^b	$N \leq 3^c$	$N > 3^d$	Mean (SE)^b	Mean (SE)^b
1999	3–16 Aug.	28	12	2	6	46	0	94	28	0.25 (0.10)	68	3	0.58 (0.11)	0.95 (0.19)
2000	31 Jul. to 15 Aug.	29	7	2	3	9	2	52	4	0.09 (0.06)	30	2	0.66 (0.20)	0.85 (0.25)
2001	25 Jul. to 10 Aug.	6	0	0	1	10	0	17	3	0.04 (0.02)	9	1	0.28 (0.18)	0.35 (0.18)
2002	6–18 Aug.	21	0	0	7	31	0	59	13	0.05 (0.01)	40	1	0.55 (0.16)	0.65 (0.18)
2003	11–25 Jul.	36	15	3	33	10	3	100	55	0.33 (0.05)	69	25	2.46 (0.34)	2.90 (0.37)
	10 Jun. to 10 Oct.	2	1	0	1	2	0	39	25	0.47 (0.17)	26	13	3.47 (0.86)	4.19 (0.92)
2004	12–26 Jul.	30	18	6	23	23	2	102	60	0.44 (0.08)	38	57	7.59 (1.10)	8.42 (1.13)

[a] No. plots with acridid density >0.
[b] Mean (SE) of the no. acridids per square meter.
[c] No. plots with acridid density >0 and <3 per square meter.
[d] No. plots with acridid density >3 per square meter.

total number of acridids, including nymphs and adults, per sample unit was determined in the field. Adults were assigned to one of three groups: *Calliptamus* sp., *Chorthippus*/*Euchorthippus*, and "other" spp.; nymphs were assigned to one of three groups: *Calliptamus* sp., *Gomphocerinae* spp., and "other" spp. To estimate the relative abundance of each species, 1 of 10 adults (between 1999 and 2003) or all individuals (2004) were collected for laboratory identification.

Numerical Sampling Design. An analysis of sample size depends on a goal for estimator precision and on an expression of sample size, *n*, as a function of estimator variance, confidence interval length, or some other measure of reliability. This requires an analytic expression for the variance of the estimate or a probability model for the estimator (Williams et al. 2002). A way to define reliability for a mean estimate is to set the SE of the mean equal to a fixed proportion (D) of the mean (μ). Hence:

$$\frac{\sigma}{\sqrt{n}} = D\mu \qquad [1]$$

where n and σ^2 are the sample size and variance of counts per sample unit, respectively.

Distribution of sample counts can be approximated by a probability distribution function. In general, variability among sample units is best approached using variance–mean relationships. Taylor's law (1961) assumes the sample mean and variance estimates are linked as $s^2 = a\bar{x}^b$, which can be linearized:

$$\log_{10}s^2 = \log_{10}a + b\log_{10}\bar{x} \qquad [2]$$

where $\bar{x}$ is the sample mean of the number of acridids in a sample of size n, and $\log_{10}a$ and b are least-squares regression coefficients (Taylor and Woiwod 1982). From equations 1 and 2, we derive the required sample size n corresponding to an a priori fixed-precision level (D_o):

$$n = \frac{a}{D_0^2}\bar{x}^{b-2} \qquad [3]$$

We calculated sample means and variances of the number of acridids per sample unit for each data set with 188 nonzero fields with *C. italicus* and 382 nonzero fields with *Chorthippus*/*Euchorthippus* (Table 1). 2000 and 2001 were omitted for *C. italicus* and 2001 was omitted for *Chorthippus*/*Euchorthippus* because of the low number of nonzero fields these years (Table 1). The original model with year as a fixed factor in equation 2 ($Y_{ij} = a'_i + b_iX_{ij} + \varepsilon_{ij}$ with $Y_{ij} = \log_{10} s_{ij}^2$, $X_{ij} = \log_{10}\bar{x}_{ij}$ for field j in year i) was compared with model described in equation 2 with no year effect ($Y_{ij} = a' + bX_{ij} + \varepsilon_{ij}$) using analysis of variance (ANOVA) (Crawley 2002). Parameters obtained for each acridid taxon were used in equation 3 to develop sampling designs for different precision levels. We also analyzed the effect of plot area (PA; ha) in 2003 and 2004 on the variance–mean relationship for the two species groups. The complete model was $\log_{10}s^2 = c + b\log_{10}\bar{x} + dPA$. We used Akaike Information Criterion (AIC) (Crawley 2002) to compare the complete model to the simplified model described with equation 2.

Binomial Sampling Design. Binomial sampling plan for estimating density can be based on empirical relationships between mean density of individuals per sample unit ($\bar{x}$) and the proportion of sample units with at least T individuals (P_T). We chose the model of Gerrard and Chiang (1970):

$$\ln\bar{x} = \alpha + \beta\ln[-\ln(1 - P_T)] \qquad [4]$$

in which parameters α and β are estimated by linear regression. The prediction variance associated with the estimation of $\bar{x}$ from P_T has been given by Schaalje et al. (1991), who decomposed it into three components (c_1, c_2, and c_3):

$$\sigma_{\bar{x}}^2 = \bar{x}^2(c_1 + c_2 + MSE - c_3) \qquad [5]$$

where MSE is the mean square error term from fitting equation 4. Defining precision as in equation 1 and substituting the approximation $\sigma_{\bar{x}}$ in equation 5 for $\frac{\sigma}{\sqrt{n}}$ gives

$$D = (c_1 + c_2 + MSE - c_3)^{1/2} \qquad [6]$$

where c_1, c_2, and c_3 and MSE are calculated from equations in Schaalje et al. (1991). In this expression, sample size, n, contributes to c_1 and c_3 and can be calculated for an a priori fixed-precision level (D_o).

We calculated sample means of the number of acridids per sample unit and the proportions of sample units occupied with at least $T = 1$ acridid for each of the data sets for which $0 < P_T < 1$. Therefore, 172 data sets were used to establish the relationship for *C. italicus* and 288 for the *Chorthippus* /*Euchorthippus* group. Linear models between years were compared with an ANOVA using the ANOVA as described above.

Time Cost of Sampling. To establish sampling time required either to detect presence–absence or to individually count all acridids within the cage sampler, we chose eight plots in 2004 among alfalfa and grasslands managed by grazing, mowing, or set aside (Table 2). To sample a range of acridid habitats, fields were selected according to crop type, area ≥2 ha, vegetation height, percentage of soil cover with visual estimates, and acridid density. Acridid populations were sampled on 1 d for each field between 19 and 23 July by randomly throwing the cage sampler 30 times in each field. After each trial, two observers sampled simultaneously for *C. italicus*, *Chorthippus*/*Euchorthippus*, and "other" spp. A third observer was available to record counts and to observe the sampling time. For each sample unit, the number of acridids and time of catch were noted. When the presumed last acridid was found, observers spent an additional 2 m searching the cage to ensure the total acridid count. Measures of time are in seconds.

To compare numerical and binomial sampling plans, we evaluated sampling costs as a function of the sample size and of the number of sample units with k acridids. The cost function for a numerical sample of size n was obtained by summing the time spent to walk and throw the cage sampler in the field (assumed at 40 s per sample unit), the time to declare that the cage samplers were acridid-free (β_0 per empty cage sampler), the time to

Table 2. Crop type, vegetation height, percentage of soil cover, observed average density (SE) of acridids per cage sampler (1 m²), and average time in seconds (SE) to catch the first acridid in a cage sampler

Field	Crop	Height (cm)	Percent soil cover	*C. italicus*		*Chorthippus/Euchorthippus*	
				Mean density	Time (first)[a]	Mean density	Time (first)[a]
1	Alfalfa	50	80	4.93 (0.63)	9.3 (2.1)	3.26 (0.46)	9.4 (1.8)
2	Alfalfa	50	80	0.23 (0.09)	5.0 (0)	2.73 (0.41)	5.7 (0.8)
3	Alfalfa	30	50	0.06 (0.04)	—	0.56 (0.20)	11.7 (2.9)
4	Alfalfa	40	90	0.06 (0.04)	—	0.66 (0.20)	8.6 (1.5)
5	Set-aside	100	6	0.50 (0.15)	15.0 (6.0)	0.83 (0.17)	7.0 (1.4)
6	Pasture	60	80	0	—	3.56 (0.63)	5.4 (0.4)
7	Meadow	20	95	0.10 (0.05)	—	34.40 (3.26)	5.0 (0)
8	Meadow	20	80	1.03 (0.22)	9.4 (1.7)	7.03 (1.66)	5.3 (0.3)

[a] Mean (SE) of the time to see the first individual in a cage sampler.

catch and count all k (k > 0) acridids in the cages, and time to declare that all individuals were recovered in the cage (C, for non-null cage sampler):

$$t_n = 40n + \beta_0 n_0 + \sum_{n} f(k) + C_{n>0} \qquad [7]$$

where n_k is the number of sample units with exactly k acridids. Function f(k) was estimated for k > 0 using linear least squares regression and C as the 95% percentile of the maximum time between two consecutive catches in a cage sampler. Functions f(k) established for the two taxons were compared using ANOVA.

Similarly, the cost function for a binomial sample of size *n* was obtained as summing time spent to walk and throw the cage samplers in the field and time to declare the cage samplers empty or not:

$$f_n = 40n + \sum_{0}^{k_{max}} \beta_k n_k \qquad [8]$$

where n_k is the number of sample units with exactly k acridids. We calculated β_k as the 95% percentile of the time to catch the first acridid in a cage sampler according to the number of acridids in the cage (k). The time to declare a cage sampler acridid-free when no acridids were present was set to the maximum time to detect the first acridid in a cage sampler, i.e., when k = 1.

Bias and Precision in the Estimates of Population Densities. We simulated numerical and binomial sampling plans with bootstrap samples, drawn from six data sets (two alfalfa plots, two set-asides, two meadows) of 30 sample units each. We used sampling with replacement. None of these data sets were used for previous estimates and analyses. We carried out 100 simulations of each sampling design. Each simulation consisted in the random choice of an initial sample of i sample units within the bootstrap sample (i = 10 for numerical sampling as suggested for sampling densities of insect populations by Kuno (1972) i = 15 for binomial sampling). An estimate of the mean for numerical sampling or of the proportion of occupied sampling units for binomial sampling was drawn from this subsample, and sample size (*n*) was calculated to reach precisions D_0. We randomly selected *n* − i additional sample units. A maximum sample size was set to 50 for numerical sampling and to 100 for binomial sampling. We calculated sampling characteristics of the numerical sampling plan, i.e., the estimated mean of this sample of size *n*, the true precision, and the sampling time according to equation 7 with estimated parameters (Tables 3 and 4). The estimated mean from the estimated proportions of occupied sample units and the sampling time according to equation 8 with estimated parameters (Table 4) were calculated to characterize binomial sampling. We calculated summary statistics from the 100 simulations of each sampling design for the estimated mean number of acridids per sample unit, the sample size, and the sampling time.

We performed simulations of the binomial sampling designs with D_0 = 0.30 and D_0 = 0.35 for *C. italicus.* The numerical sampling design for *Chorthippus/Euchorthippus* and for *C. italicus* was simulated with D_0 = 0.10, D_0 = 0.20, and D_0 = 0.30.

We used software Splus (2004) for all statistical analyses and simulations.

Results

Numerical Sampling Design and the Variance–Mean Relationship. Density ranged between 0.1 and 7.7/m² for *C. italicus* and between 0.1 and 60.5/m² for *Chorthippus/Euchorthippus.* Regression statistics for the relationship between $\log_{10}s^2$ and $\log_{10}\bar{x}$ were es-

Table 3. Regression statistics for the linear regression between the time spent per cage sampler to catch the k acridids captured in the cage sampler, average maximum time (SE) between two captures, and C

Acridid taxon	α	SE (α)	β	SE (β)	R^2	RSE	df	Time[a]	C (95%)[b]
C. italicus	11.016	1.801	2.631	0.125	0.65	24.12	240	14.57 (2.45)	40 (*n* = 35)
Chorthippus/Euchorthippus								11.78 (1.03)	30 (*n* = 141)

[a] Mean (SE) of the maximum time between the capture of two acridids in a cage sampler (in seconds).
[b] 95% percentile of the maximum time between the capture of two consecutive acridids in a cage sampler.

Table 4. Average (SE) and range of time in seconds to catch the first acridid in a cage sampler number of sample units with acridids and estimated parameters for the time function of binomial sampling design (β_k)

Acridid taxon	Number of acridids in the cage sampler (k)				
	1	2	3	4	>4
C. italicus					
Mean (SE)	15.3 (2.7)	11.7 (3.3)	5 (0)	5 (0)	5 (0)
n_k	36	6	8	7	14
Range	5–55	5–25	5	5	5
Time (β_k)[a]	45	25	5	5	5
Chorthippus/Euchorthippus					
Mean (SE)	9.7 (1.3)	8.2 (1.3)	5.4 (0.4)	5.9 (0.9)	5 (0)
n_k	32	38	26	11	66
Range	5–25	5–45	5–15	5–15	5
Time(β_k)[a]	25	25	5	5	5

[a] 95% percentile of the time required to catch the first acridid in a cage sampler.

tablished separately for *C. italicus* and *Chorthippus/Euchorthippus.* The effect of year was not significant for parameters a and b of Taylor's power law for *C. italicus* ($F = 0.69$; df = 176,182; $P = 0.65$), and thus a single regression model was used combining all years (Table 5). Plot area, which ranged between 0.2 and 12.3 ha, had no significant effect (ΔAIC[complete versus simplified] = 1.78) on sample variance of *C. italicus.* Conversely, parameters a and b significantly differed among years for *Chorthippus/Euchorthippus* ($F = 4.62$; df = 362,370; $P < 0.01$) because of particularly high densities (>60/m^2 in some fields) in 2004 (Table 1). Data were split into two groups based on average density (≤3/m^2 or >3/m^2) and analyzed separately; densities >3/m^2 only occurred in 2003 and 2004. There was no year effect on parameters a and b either for densities ≤3 ($F = 1.53$; df = 262,270; $P = 0.15$) or for densities >3 ($F = 1.58$; df = 90,92; $P = 0.22$). The variance–mean relationship was not affected by plot area (ΔAIC[complete versus simplified] = 1.82 for grasshopper densities ≤ 3/m^2 and ΔAIC[complete versus simplified] = 1.67 for grasshopper densities > 3/m^2).

Using equation 3 and parameters estimated above (Table 5) for *C. italicus* and for *Chorthippus/Euchorthippus*, we derived a sample size for $D_0 = 0.10$, $D_0 = 0.20$, and $D_0 = 0.30$. Obtained sample sizes were similar between the two taxons except when the desired level of precision was $D_0 = 0.10$ and when density was >10/m^2. In that case, about eight more sample units were required for *Chorthippus/Euchorthippus* than for *C. italicus.* The estimated sample sizes were >50 sample units for acridid densities <1/m^2 (Fig. 2), whatever the precision, and decreased to 25–40 sample units when densities ranged between 5 and 15/m^2 for $D_0 = 0.10$. Our current standard monitoring sample size of 10 u therefore leads to expected precisions of 0.20–0.30 for intermediate densities (2.5–6 *C. italicus*/m^2 and 3–8 *Chorthippus/Euchorthippus*/m^2) and to precision better than 0.20 when densities are above 6 *C. italicus*/m^2 and 8 *Chorthippus/Euchorthippus*/m^2.

Binomial Sampling Design and Relationship Between Mean and the Proportion of Units with Acridids. Parameters α and β were not affected by year (Gerrard and Chiang model) for *C. italicus* ($F = 1.69$; df = 164,170; $P = 0.13$); therefore, a single regression equation was used for all years (Table 6). The different variance components and precision values were calculated for P_T and ranged from 0.10 to 0.90 for three sample sizes ($n = 15$, $n = 40$, and $n = 100$). The highest precision values were obtained when $P_T = 0.5$, where D = 0.35, 0.30, and 0.28 for sample sizes n = 15, 40, and 100, respectively. Thus D = 0.28 was set as the best possible achievable precision in our binomial sampling designs. The relationships differed among years for *Chorthippus/Euchorthippus* ($F = 3.01$; df = 278,286; $P < 0.01$) because of a high infestation in 2004. On the contrary, values of parameter β did not differ among years (Table 6). Even if we split data sets according to density, intercept values remained different between years, and therefore, we could not calculate a binomial sampling design for *Chorthippus/Euchorthippus.*

We used equation 6 and the estimated parameters of the Gerrard and Chiang relationship (Table 6) to calculate the number of sample units required for sampling *C. italicus* on the basis of binomial sampling designs with a precision level D_0. The sample size was at least 54 sample units for the level of precision $D_0 = 0.30$ (Fig. 3), and it was obtained for an occupancy rate $P_T = 0.5$. The level of precision $D_0 = 0.35$ led to sample sizes that ranged from 16 to 50 sample units according to *C. italicus* densities (Fig. 3B). The range of densities

Table 5. Regression statistics for relationship between logarithm of variance and logarithm of mean of acridid counts per square meter ($\log_{10}s^2 = \log_{10}a + b\log_{10}\bar{x}$)

Acridid taxon	Subsample	$\log_{10}a$[a]	SE ($\log_{10}a$)	b	SE (b)	R^2	RSE	df
C. italicus	All years	0.202	0.015	1.229	0.023	0.94	0.146	179
Chorthippus/Euchorthippus	Density: 0.1–3/m^2	0.156	0.014	1.193	0.027	0.87	0.198	270
	Density: >3/m^2	0	0.08	1.559	0.092	0.75	0.281	92

[a] $a = 10^{\log_{10}a - 0.5SE(\log_{10}a)}$ (Goldberger 1968).

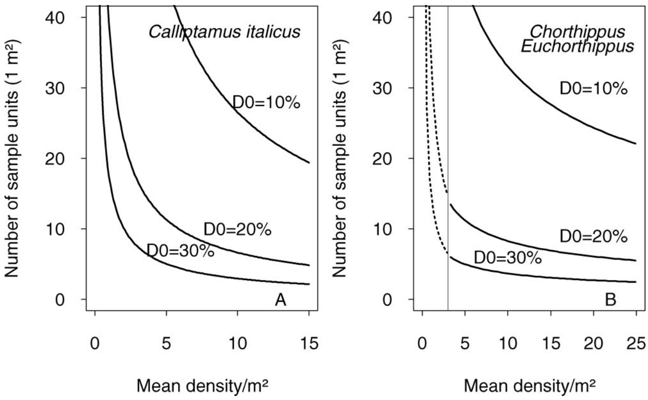

Fig. 2. Numerical sampling design for estimating density at three levels of relative precisions D_0: (A) *C. italicus* and (B) *Chorthippus/Euchorthippus.*

that is possible to sample before saturation is from 0 to ≈4 *C. italicus* per sample unit (Fig. 3B), and such densities correspond to occupancy rates from 0.13 to 0.95 (Fig. 3A).

Time Needed for Sampling Counts or Presence–Absence Data. Time to detect the first individual in the cage sampler was related to the number of individuals in the cage, whatever the taxon (Table 4). On average, 15.3 s were required to catch the first *C. italicus* when it was alone in the cage, but it was reduced to ~5 s when at least two *C. italicus* were in the cage. The first *Chorthippus/Euchorthippus* was detected in 9.7 s when it was alone (Table 4). We established the 95 percentile of these times to estimate parameters β_k for binomial sampling design, leading to the establishment of three density categories for *C. italicus* and two for *Chorthippus/Euchorthippus* (Table 4). Time to find all the individuals within a cage sampler was again related to the number of individuals in the cage (Table 3), but similar parameters were estimated for the two taxons (F = 1.50; df = 239,1; P = 0.22). Within the cage sampler, the maximum time elapsed between consecutive captures of individuals could reach 1 m, but on average, it was 10–15 s (Table 3). We calculated the 95 percentile of these maximum times to estimate the time required, C, before declaring that there were no more individuals in the cage sampler. The C–value varied according to the species (Table 3) and was longer for *C. italicus* than for *Chorthippus/Euchorthippus.*

Bias and Precision in Mean Density Estimates and Sampling Cost. The mean density of *C. italicus* in the six validation plots ranged from 0.44 to 5.11/m^2 (Fig. 4A), and the proportion of sample units occupied by at least one individual ranged from 0.32 to 1. Summary statistics of the 100 simulation runs per numerical sampling design and per validation plot showed that the average estimated means were actually very close to the plot sample means (Fig. 4A). The 95% simulated CIs for the estimated means increased with D_0 and was highest for plot P6, which had the highest *C. italicus* density (Fig. 4A). Sampling was truncated at the maximum sample size for all data sets except P6 when the required precision was set to D_0 = 0.10 (Fig. 4C), and for this reason, the levels of precision actually reached were less than the desired precision. In the other cases, the required precisions were reached for all plots except P4 (Fig. 4B). When the level of precision was set at D_0 = 0.20 and D_0 = 0.30, sample sizes ranged between 10 and 24, when *C. italicus* density was >1/m^2. *C. italicus* densities <1/m^2 required >40 sample units to reach D_0 = 0.20. Time spent to sample the validation plots ranged between 20 and 80 m (Fig. 4D) depending on *C. italicus* density and the required level of precision. Simulations of the binomial sampling design for D_0 = 0.30 and D_0 = 0.35 (Fig. 5A) indicated that this sampling design was not possible for P6 because the occupancy rate was too close to 1 (Fig. 5B). In the other cases, the average estimated means were very close to the sample means of the validation plots. The simulated 95% CIs for mean estimates were smaller than those obtained when numerical sampling design was simulated. Sample sizes were large (Fig. 5C) when we set D_0 = 0.30 and ranged between 64 and 75 sample units, whereas when we set the desired level of precision to D_0 = 0.35, sample sizes ranged from 18 to 25 u. Time spent to sample the plots was important (Fig. 5D) for the best required precision D_0 = 0.30, whereas it was ≈30 m when we set D_0 = 0.35.

The mean density of *Chorthippus/Euchorthippus* from validation plots ranged from 0.84 to 17.33 grasshoppers/m^2 (Fig. 6A). Summary statistics of the 100

Table 6. Regression statistics for the linear regression of $\ln \bar{x} = \alpha + \beta \ln[-\ln(1 - P_T)]$

Acridid taxon	Subsample	α	SE (α)	β	SE (β)	R^2	RSE	df
C. italicus	1999, 2002–2004	0.229	0.038	1.091	0.025	0.92	0.276	170
Chorthippus/Euchorthippus	1999, 2000, 2002–2004	0.181	0.026	1.068	0.021	0.91	0.311	236
	2004	0.419	0.058	1.062	0.066	0.84	0.412	48

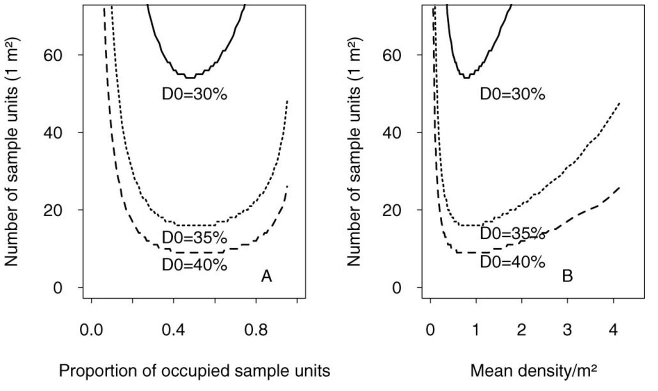

Fig. 3. Binomial sampling design for estimating density of *C. italicus* at three levels of relative precisions D_0: (A) sample size related to cage sampler occupancy rate and (B) sample size related to density.

simulation runs obtained from the numerical sampling design (Fig. 6A) showed that the average estimated means were very close to the observed means of the validation plots. The 95% simulated CIs for the estimated means increased with D_0 and with grasshopper density (Fig. 6A). The levels of precision were better than the desired levels of precision in 15 of 18 cases (Fig. 6B). When $D_0 = 0.10$, sample sizes ranged from 32 (highest density) to 50 (smallest density; Fig. 6C). When density was $>2/\text{m}^2$, 15 sample units were enough to reach $D_0 = 0.20$. Time spent to sample grasshoppers in the validation plots was ≈20 m (Fig. 6D) except when we required the best precision and when grasshopper density was $<1/\text{m}^2$.

Discussion

Binomial Versus Numerical Sampling for Estimating *C. italicus* Density. *Calliptamus italicus* is of particular interest because it can be a solitary or gregarious locust, can use a wide range of habitats, and is found throughout Middle Asia, Minor Asia, Iran, and Southern Europe (Uvarov 1977). Populations of *C. italicus* can build up to outbreak densities and cause considerable damage (Louveaux et al. 1988, Stolyarov 2000). Although collected every year of our study, the occurrence ranged from 8 to 64% of grassland fields surveyed (Table 1). We found that population mean density was related to variance by a power function, as in many other animal species (Taylor 1961, 1984). Parameter b of Taylor's power law was regarded by Taylor (1961) as an "index of aggregation," being characteristic for each species. In our study, mean densities of *C. italicus* per square meter ranged from 0.1 to $7.7/\text{m}^2$, and count variability was not affected by the area of the field. We found a very good fit to Taylor's linear regression ($r^2 = 0.94$), and the estimate of b was b = 1.229 (parameters a and b were identical in the 4 yr of the study). These observed densities are quite low and are typical of nonswarming populations (Uvarov 1977). These estimated parameters should

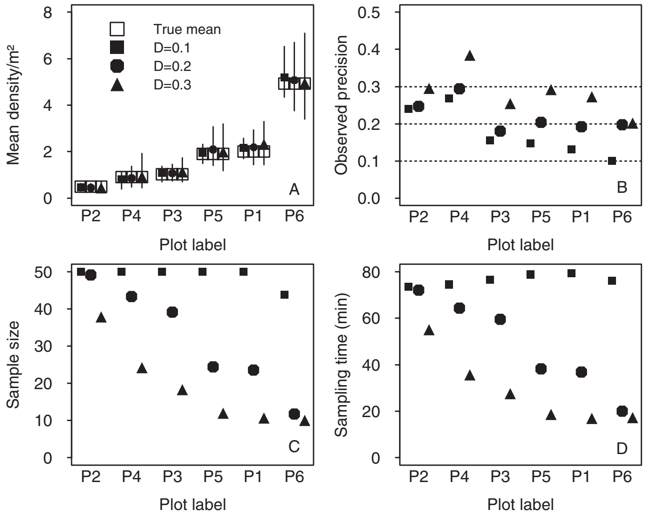

Fig. 4. Simulation of the numerical sampling design for estimating density of *C. italicus* on validation plots (P1–P6) at three levels of relative precisions (D_0). (A) Estimated means in black compared with the validation plot mean in white. (B) Precisions really reached calculated as $D = s/\sqrt{n}$. (C) Sample size. (D) Sampling time.

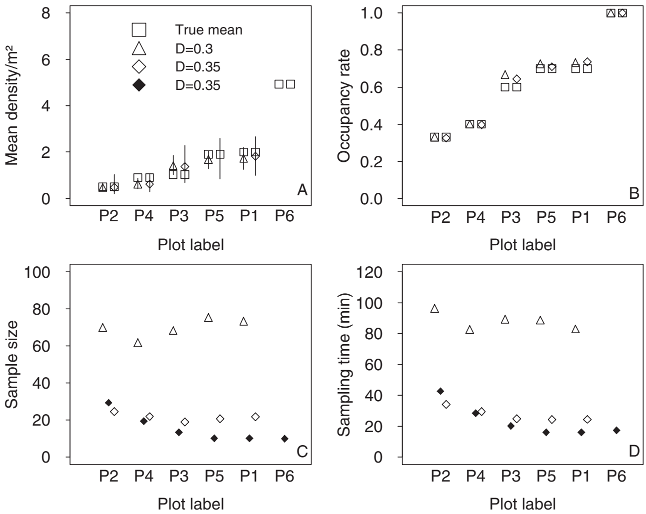

Fig. 5. Simulation of the binomial sampling design for estimating density of *C. italicus* on validation plots (P1–P6) at two levels of relative precisions (D_0). (A) Estimated means (white triangle and white diamond) compared with the validation plot mean (white square). (B) Occupancy rates. (C) Sample sizes. (D) Sampling times. Sample sizes and sampling times simulated with numerical sampling design with D_0 set to 0.35 are added for comparison (black diamond).

therefore not apply for gregarious populations that exhibit very different spatial behavior and higher densities (Uvarov 1977; but see Tokeshi 1995 and Hanski 1999). For required levels of precision at $D_0 = 0.20$ or $D_0 = 0.30$, estimated sample sizes ranged between 10 and 20 sample units, which is the sample size we currently use for our standard monitoring. Simulations of these numerical sampling plans further indicated that they lead to unbiased estimates and required precision. However, variability in density estimates was high (Fig. 4A), whatever the validation plot, and this was also the case for the level of precision really achieved. Variability in mean estimates was already underlined by Hutchison et al. (1988) and Naranjo and Flint (1995), and was associated with the sampling process (Anscombe 1952) and the spatial dispersion parameters a and b, which may be influenced by environmental and climatic factors (Taylor 1984).

The relationship between mean and the proportion of occupied units with at least one *C. italicus* was well modeled using the model of Gerrard and Chiang ($r^2 = 0.92$), with no year effect. However, the proportion of occupied sample units was saturated, i.e., $P_1 = 1$, when the density exceeded 7/m^2. Legg et al. (1993) used 0.1-m^2 wire hoops and did not achieve saturation until 200 acridids/m^2. The best possible relative precision that

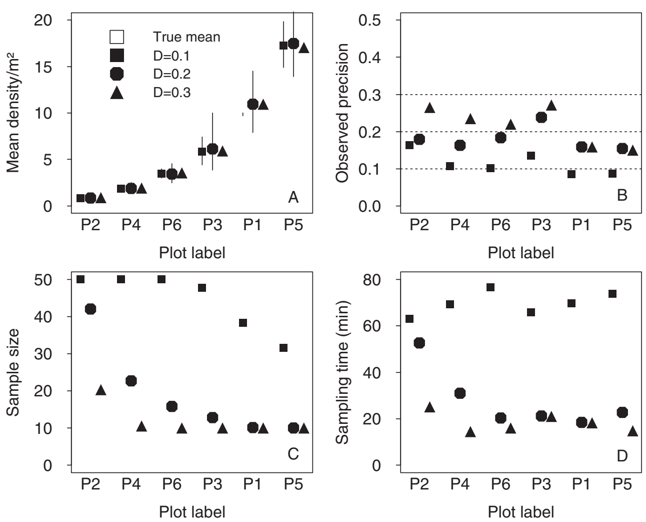

Fig. 6. Simulation of the numerical sampling design for estimating density of *Chorthippus*/*Euchorthippus* on validation plots (P1–P6) at three levels of relative precisions (D_0). (A) Estimated means in black compared with the validation plot mean in white. (B) Precisions really reached calculated as $D = s/\sqrt{n}$. (C) Sample size. (D) Sampling time.

can be reached when predicting $\bar{x}$ from P_T estimated in the field will never exceed $D_0 = 0.28$. Such results are more precise than those obtained by Naranjo et al. (1996) for the leafhopper *Bemisia tabaci* Gennadius and by Zhang et al. (1996) for the green peach aphid, *Myzus persicae* Sulzer, who did not achieve a precision better than 0.42 and 0.87, respectively. Legg et al. (1993) obtained similar prediction errors for $\bar{x}$ (0.33) when using the Nachman model (a model similar to the Gerrard and Chiang model) for estimating acridid densities in New Mexico. For $D_0 = 0.30$, sampling time when using binomial sampling strategy was more than twice when using numerical sampling strategy (Figs. 4 and 5), but for $D_0 = 0.35$, sampling times were equivalent with the two procedures (Fig. 5D) except for one plot. In conclusion, binomial sampling can be recommended when densities are low ($<2/m^2$) and when the required precision is above 0.35, or alternatively, if the purpose of population monitoring is qualitative. For more precise sampling and/or high *C. italicus* densities, numerical sampling performs better, provided that the plot area is large enough to withstand disturbance causd by sampler movements.

Numerical Sampling for Estimating Density of *Chorthippus* spp. and *Euchorthippus* spp Grasshoppers. *Chorthippus* spp. are dominant in European grasslands (Baldi and Kisbenedek 1997, Kruess and Tscharntke 2002, Reinhardt et al. 2005) including France (Voisin 1979, 1990). In our study site, five species of *Chorthippus* (*C. albomarginatus* De Geer, *C. biguttulus* L., *C. brunneus* Thunberg, *C. dorsatus* Zetterstedt, and *C. parallelus* Zetterstedt) and two species of *Euchorthippus* (*E. elegantulus* Zeuner, and *E. declivus* Brisout) were captured (Badenhausser and Bretagnolle 2005). These species were present whatever the year (Table 1), and the range of their densities varied from 0.1 to 60.5 individuals/m^2. They were present every year in 59–100% of the samples, with an average of 80%. Conversely to *C. italicus,* the variance–mean relationship varied with years and densities (the two effects could be confounded). It is known that acridids respond differently to intraspecific and interspecific competition, in relation to food limitation (Evans 1992, Joern and Klucas 1993, Fielding 2004), so that the community structure (species and relative abundance) and the spatial pattern may vary with density. Furthermore, the structure of acridid communities is influenced by management and vegetation parameters, which are both controlled by human activities (Guido and Gianelle 2001, Kruess and Tscharntke 2002, Wingerden et al. 1992). In our study, the relative abundance of each of the seven species mentioned above differed between years. Dominant species were *C. biguttulus* and *E. elegantulus* every year, and their relative abundances ranged for *C. biguttulus* from 15.9% in 2004 to 48% in 2002 and for *E. elegantulus* from 41.3% in 2002 to 71.4% in 2001. In contrast, some species were not captured or were rare in some years (*C. parallelus,* 0% in 2001; *C. albomarginatus,* 0.5% in 2003), but were abundant in other years (*C. parallelus,* 21.3% in 2004; *C. albomarginatus,* 12.2% in 2004). Numerical sampling plan derived from variance–mean relationships lead to sample sizes that were compatible with practical constraints when $D_0 = 0.20$ or $D_0 = 0.30$. Simulation studies on our validation data sets further showed that, with this sampling plan, estimates were unbiased whatever the level of required precision (Fig. 6C) and that the observed precisions were close to the required precisions (Fig. 6D). Moreover, we showed that there was no need to take into account the plot area for determining sample size because count variability was not related to plot area within the observed range (0.2–12.3 ha). Sampling plans based on binomial counts were not established because intercept α in the model of Gerard and Chiang systematically differed among years. As for *C. italicus,* the proportion of occupied samples was saturated when the density exceeded 6–7 acridids/m^2, so that it could not have been used for higher densities. Therefore, we advocate again for numerical sampling design for *Chorthippus/Euchorthippus* for all density estimations.

Effective sampling design to estimate acridid density per surface area unit in grassland habitats can be used in all field area and should be based on insect counts for *Chorthippus/Euchorthippus,* whereas it could be based either on presence–absence data or count data for *C. italicus* according to insect density and precision.

Acknowledgments

We thank C. Attié, L. Bruneteau, S. Jaulin, S. Lorioux, A. Morel, and M. Vandier for technical assistance in the field, J. Lerin for useful comments on the manuscript, and M. Lemonnier-Darcemont and C. Darcemont for help at the early stage of this work in acridid identification. Financial support for this study was provided by DIREN Poitou-Charentes, Ministère de l'Ecologie et du Développement Durable (Programme DIVA) and INRA (Programme ECOGER).

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Received for publication 2 November 2006; accepted 13 August 2007.