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Spatial Distribution and Fixed-Precision Sequential Sampling of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) on Cucumber Greenhouse

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Abstract: The spatial distribution of larvae of *Liriomyza sativae* Blanchard on greenhouse cucumber leaves was studied. Sampling was performed once a week at an experimental greenhouse located in Jiroft (Kerman, Iran) during the growing seasons of 2008 and 2009. The spatial distribution of larvae of the leafminer, was described by calculation of different dispersion indices. The within- greenhouse spatial patterns of larvae were aggregated and the estimated Taylor and Iwao indices (b and β) have been ranged from 1.174 to 1.317 and 1.154 to 1.231 respectively. To estimate the density of *L. sativae* larvae, a fixed-precision sequential sampling plan was developed using the parameters of Taylor's power law generated from total number of larvae in a cucumber leaf at two precision levels (D) of 0.1 and 0.25. Regarding required sample size, the developed fixed-precision sequential sampling plans showed an acceptable performance for estimating leafminer density at the precision levels of 0.1 and 0.25. Optimum sample size was flexible and depended upon the leafminer density and desired level of precision, it ranged from 3 to 197 and 15 to 1229 leaves at the precision levels of 0.25 and 0.1 respectively.

Key words: Vegetable leafminer · Sampling · Cucumber greenhouse · Ecological studies · Sequential sampling

INTRODUCTION

Leafminers belonging to the genus *Liriomyza* (Diptera, Agromyzidae) are regarded as pests in many crops due to their damage to leaves [1]. The genus *Liriomyza* includes about 300 species distributed worldwide with 23 species being considered economically important [2, 3]. The leaf miner fly, *Liriomyza sativae* Blanchard, originated from the Neotropics, was reportedly seen in Mexico and South America, but has rapidly disseminated to other countries in Europe, Africa and Asia [1]. In Iran, *L. sativae* was first seen in the year 2000. This species and *L. trifolii* Burgess have seriously damaged beans, peas, vegetables and tomatoes in the provinces of Khuzestan, Kerman and Tehran [4-6]. At present, *L. sativae* mixed with *L. trifolii* which is mostly dominated by *L. sativae* in cucumber greenhouses throughout the country.

As a polyphagous insect, *L. sativae* affects many host plants including horticultural crops and all associated weeds [1]. Flowering plants which are readily infested and are known to facilitate the spread of the pest include chrysanthemum, gerbera, gypsophila and marigold, but there might be many other hosts, especially among Compositae [7].

Leaf miners have a relatively short life cycle; they are able to complete their development in 21-28 days under warm environments such as Florida and Egypt (Dimetry, 1971). In tropical climates, numerous generations occur annually [8]. Leibee [9], determined growth at a constant 25°C and reported that about 19 days were required from egg deposition to emergence of the adult.

The management of agromyzid leaf miners has been a topic of extensive research and scientific debate for the last three decades. Most of studies have focused on using synthetic and natural insecticides, which are commonly used similarly by both the small holder farmers and large-scale producers. However, their effectiveness has been doubted due to their broad-spectrum application, the impact on natural enemies and the development of resistance in target pests. Other control techniques, such as using yellow sticky traps or resistant host plants, currently have a very limited usage in some countries [10].

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Spatial distribution is a behavioral response of the individuals of a species to habitat [11, 12]. The information of special distribution (i.e., regular, random or aggregated) can determine what sampling program must be carried out, especially sequential sampling [13, 14].

A successful management of leafminers strongly depends on the development of an appropriate sampling plan (i.e., easy to implemented suitable for rapid decision-making processes). In sampling programs, precision and cost-effectiveness are two most important factors that need to be considered [15]. For example, compared with fixed-sample size sampling, a fixed-precision sequential sampling can result in a 35-50% reduction in sampling effort [16]. The development of a sequential sampling scheme with a fixed statistical precision, therefore, may be useful for estimating *L. sativae* density in cucumber greenhouses, which in turn, would be valuable for ecological and pest management studies.

The objectives of the present study were to determine the spatial distribution patterns for *L. sativae* larva and to develop and evaluate a fixed-precision sequential sampling for estimating leaf miner densities in cucumber greenhouses.

MATERIAL AND METHODS

The Study Site: Field experiments were carried out at an experimental greenhouse located in Jiroft (Kerman, Iran) during growing seasons (November- April) in 2007-2009. The cucumber *Cucumis sativus* cv. RS189 I SINA F1 (Royal Sluis, Netherlands) was grown under greenhouse on eight 45-m-long rows. Cultivations, fertilization and irrigations were conducted according to the conventional agronomic practices. No other pesticides were applied.

Sampling Unit: One single leaf of a cucumber plant was randomly selected as a sample unit. Then, it was inspected by stereomicroscope to determine the number of larvae of *L. sativae* per leaf.

Sampling Pattern and Timing: Cucumber leaves were randomly sampled and counted for larval density of *L. sativae* once a week during morning.

Sample Size: Primary samples were taken in a random number of leaves. The reliable sampling size was determined using the following equation:

$$N = \left(\frac{ts}{dm}\right)^2$$

Where N, t, s, d and m are sample size, t-student, standard deviation, desired fixed proportion of the mean and the mean of primary data, respectively [17].

Relative variation (RV) was used to compare the efficiency of various sampling methods [18]. The RV was calculated as the following:

$$RV = \left(\frac{S_E}{m}\right)100$$

Where S_E and m are the standard error of the mean and the mean of primary sampling data, respectively.

Spatial Distribution

Distribution Indices

Index of Distribution: Distribution of population was classified using calculation of the variance to mean ratio (S^2 / m). Departure from the random distribution was then tested by calculating the index of distribution (I_d), as follows:

$$I_d = (n-1)\frac{S^2}{m}$$

Where n denotes the number of samples [17].
This index was tested by Z values as following:

$$Z = \sqrt{2I_d} - \sqrt{(2v-1)} \text{ and } v = n-1$$

Morisita's Coefficient of Distribution: The uneven distribution coefficient (I_a) was calculated through the following equation:

$$I_\delta = \frac{n\sum xi(xi-1)}{N(N-1)}$$

Where n, x_i and N are the number of sample units, the number of individuals in each sample unit and total number of individuals in n samples, respectively. To determine whether the sampled population was significantly different from random distribution; the large sample test of significance was applied using Z values as follows:

$$Z = \frac{I_\delta - 1}{\left(\frac{2}{nm^2}\right)^{1/2}}$$

Where m and n are the mean population density per leaf in each sampling date and the number of sample units, respectively [19].

Taylor's Power Law: Taylor's power law was calculated as following:

$$S^2 = \alpha m^b \text{ or } \log S^2 = \log a + b \log m$$

Where the parameters a and b are a scaling factor related to sample size and an index of aggregation, respectively [17].

Iwao's Patchiness Regression Models: Iwao's patchiness regression method was applied to quantify the relationship between mean crowding index (m^*) and mean (m) using the following equation:

$$m^* = \alpha + \beta m$$

Where α and β refer to the tendency to crowding/repulsion and the distribution of population on space.

The values of F and P acquired from regression equations were used to test whether the Taylor's (b) and Iwao's (β) coefficients were significantly different from 0. In addition, to test for their difference from1, the statistic t (as $t = (slope - 1)/SE_{slope}$ was used. Here, *slope* and SE_{slope} are Taylor's or Iwao's coefficient and their standard errors in regression equations, respectively.

Since Taylor's and Iwao's coefficients were estimated by two-year data, the difference between years' distribution coefficients, were tested by the statistic t ($t = \frac{b_1 - b_2}{\sqrt{SE_1^2 + SE_2^2}}$) [20, 21]. Here, b_1(and SE_1) and b_2(and SE_2) are the Taylor's or Iwao's coefficient (and its standard error) for the first and the second year, respectively.

The data of two years were integrated and a total distribution coefficient was estimated only when the difference between coefficients of two years was not significant.

Spatial Distribution Models: Among various models of spatial distribution, distribution of population in each growing season was matched with Poisson and negative binomial distributions. The probability of the presence of a given number of an insect in a sample of population (x) was estimated by $P_X = \left(\frac{a^x e^{-a}}{x!}\right)$ for Poisson distribution and by $P_X = \left(\frac{1}{q^k}\right)\left(\frac{(k)(k+1)...(k+x-1)}{x!}\right)\left(\frac{p}{q}\right)^x$ for negative binomial distribution [12]. Here, a, x and $x!$, are population mean, expected population number and factorial in a sample unit (leaf), respectively. The parameters p (=X/k), q (=$p+1$) and k indicate aggregation level in negative binomial distribution. The value of k (also known as aggregation index) was estimated by $N \ln\left(1 + \frac{X}{\hat{k}}\right) - \sum\left(\frac{A_x}{\hat{k}} + x\right) = 0$. Here, N, X and A_x are the sample number, the population mean and the total observed frequencies in sampling units which had more than x individuals, respectively [17].

Sequential Sampling Planning: Green's [22] model was used for designing a sequential sampling plan with the precision levels of 0.1 and 0.25. The required sample number for estimating mean population was estimated by $n = \frac{ax^{(b-2)}}{D^2}$ and decision lines were estimated by $T_n = \left(\frac{an^{1-b}}{D^2}\right)^{\frac{1}{(2-b)}}$ [19]. Here, T_n, N, n and D are cumulative total for sample n, maximum number of sampling units, sample size and the fixed level of desired precision in terms of *SE/x*. The parameters a and b were determined from Taylor's power law [17].

RESULTS AND DISCUSSION

Sampling Program: The results of primary sampling showed that the reliable sample size with maximum variation of 20% was 37 and 25 for 2007-2008 and 2008-2009 growing seasons, respectively. The relative variation (*RV*) of the primary sampling was 11.5 and 11.12 for the previous growing seasons, respectively. These *RV*s were very appropriate for the sampling program (Table 1).

Spatial Distribution

Distribution Indices: The Taylor's equations for the growing seasons were obtained as $\log S^2 = 0.295 + 1.174 \log m$ ($F_{23} = 324.2$, $P < 0.05$; Table 2) and $\log S^2 = 0.264 + 1.317 \log m$ ($F_{20} = 313.9$, $P < 0.05$), both with a great degree of fit (> 0.90). In addition, the coefficient b was significantly greater than 1 (2007-2008: $t_{23} = 2.76$, $P < 0.05$; 2008-2009: $t_{20} = 4.28$, $P < 0.05$; Table 2), implying an aggregated distribution.

The Iwao's equation for the growing seasons were obtained as $m^* = 0.865 + 1.154m$ ($F_{23} = 429.989$, $P < 0.05$; Table 3) and $m^* = 0.604 + 1.231m$ ($F_{20} = 702.934$, $P < 0.05$), both with a great degree of fit (> 0.90). In addition, the coefficient β was significantly greater than 1 (2007-2008: $t_{23} = 2.75$, $P < 0.05$; 2008-2009: $t_{20} = 5.02$, $P < 0.05$; Table 3), implying an aggregated distribution.

Table 1: Estimated parameters from primary sampling of *Liriomyza sativae* on cucumber during 2007-2009

Growing season	n[a]	S_e[b]	Sd[c]	RV[d]	m[e]	d[f]	N[g]
2007-2008	30	0.03	0.16	11.5	0.26	0.20	37
2008-2009	30	0.02	0.09	11.12	0.18	0.20	25

[a]Number of samples; [b]Standard error of the mean; [c]Standard deviation;

[d]Relative variation; [e]Mean of primary data

[f]Desired fixed proportion of the mean, [g]Sample

Table 2: Spatial distribution of *Liriomyza sativae* on cucumber using Taylor's power law regression analysis

Growing season	b ± SE	Loga ± SE	R^2	F	t	Df
2007-2008	1.174±0.063	0.295±0.030	0.957	344.212**	2.76*	23
2008-2009	1.317±0.074	0.264±0.066	0.940	313.974**	4.28*	20
Overall	1.263±0.051	0.272±0.036	0.954	607.279**	5.15*	44

*and** show significant difference at 0.05 level with 0 and 1, respectively

Table 3: Spatial distribution of *Liriomyza sativae* on cucumber using Iwao's patchiness regression analysis

Year	β± SE	α± SE	R^2	F	t	Df
2007-2008	1.154±0.056	0.865±0.197	0.949	429.898**	2.75*	23
2008-2009	1.231±0.046	0.604±0.150	0.912	702.934**	5.02*	20
Overall	1.208±0.030	0. 48±0.274	0.932	1607.386**	6.93*	44

*and** show significant difference at 0.05 level with 0 and 1, respectively

Due to the higher precision of Taylor's coefficient, it was used for estimating spatial distribution and for designing sequential sampling plans. Furthermore, Taylor's index does not change by environmental fluctuations [23] or sample size [24]. There was no significant difference (Taylor's coefficients: t_{slope} =1.47, $t_{intercept}$ = 1.87, P <0.05; Iwao's coefficients: t_{slope} = 1.06, $t_{intercept}$ = 1.05, P <0.05) between the annual distribution coefficients. Therefore, the annual data was pooled between years and overall distribution coefficients were used (Tables 2 and 3).

Previous studies have been stated an aggregated form for the spatial distribution pattern of *Liriomyza* spp. [25-28]. Here, the estimated Taylor index *b* was between1.17 and 1.32. In other studies, the estimated values of this index has been ranged from 1.12 to 1.62, for example 1.12 on lettuce [29], 1.15 and 1.19 for *L. trifolii* mines and larvae on chrysanthemum [30], 1.16 for *L. sativae* on beans (31), 1.19 for *L. huidobrensis* larvae on celery [32], 1.51 for *L. trifolii* larvae on celery [33] and 1.62 for *L. trifolii* mines on greenhouse tomatoes [28].

In this study, the estimated Iwao index β was between1.15 and 1.24. In another study, the estimated values of this index for mines, larvae and total were 1.036, 1.084 and 1.039 respectively. To explain these differences, some researchers believe that the spatial distribution of *Liriomyza sp* on tomato leaves is more aggregated than on other host plants [28]. But considering the results of similar studies in various parts of the world, it might be concluded that the differences are at least partly caused by the different host plants, pest population density and environmental conditions such as weather, greenhouse ventilation and pesticide applications [25, 26, 29].

Similar to Taylor and Iwao indices, with Morista coefficient and I_d spatial distribution was aggregated in most sampling dates (Table 4). The estimation of Taylor and Iwao indices requires measuring mean or other population parameters during growing season. On the contrary, variance/mean ratio and Morista indices can be calculated for all sampling dates [34]. Furthermore, Morista index is not affected by sample size [35, 36]. The different distribution form of sampling dates indicate that insect's behavior varies during growth season; as population density increases the population distribution tend toward aggregation [34, 37, 38].

Spatial Distribution Models: When the spatial distribution models were fitted on the population density of *L. sativae* larvae, negative binomial model showed the best fit (2007-2008: $\chi^2 = 7.58$, $P>0.95$; 2008-2009: $\chi^2 = 13.02$, $P> 0.95$ Table 5). These results fully support the findings of distribution indices, indicating an aggregated distribution. Although distribution indices showed random distribution in some planting dates, total population distribution during growing season matched the aggregated model. The aggregated distribution of a population is likely to be confirmed by aggregated indices, but its frequencies might not be correspondent to negative binomial distribution. Further studies are needed to evaluate *L. sativae* population distribution fitting is by

Table 4: The fitted percentage of spatial distribution of *Liriomyza sativae* larvae

	Dispersion indices					
	Morista		S^2/m		I_d	
Growing season	Random	Aggregated	Random	Aggregated	Random	Aggregated
2007- 2008	36.0	64.0	0.0	100.0	28.0	72.0
2008- 2009	38.9	69.1	31.8	68.2	35.4	64.6

Table 5: The spatial distribution models fitted on *Liriomyza Sativae* larvae

	Distribution models			
	Negative binomial		Poisson	
Growing season	χ^2	Df	χ^2	Df
2007-2008	7.58	18	3853.00*	13
2008-2009	13.02	26	109698.45*	18

* shows a significant difference at 0.05 level

other aggregated distributions than negative binomial distribution such as Thomas, Neyman types A and B and logarithmic distribution. In this case, the recommendations about the kind of spatial distribution and proper test for their fitting are more reliable [34].

Sequential Sampling: Mean numbers of larvae per leaf ranged from 0.3 to 10.23 in 2008 and from 0.07 to 29.1 in 2009. With the precision of 0.25, the number of samples required for estimating the population density of *L. sativae* larvae varied between 3-197 leaves, when the mean larval density per leaf declined from 29.1 to 0.07. However, these values for population ecology studies, which need a precision of 0.1, would increase to a range of 15-1229 leaves under the same larval densities (Fig. 1).

Fixed- precision sequential sampling stop lines were calculated at two levels of precision (Fig. 2). Utilization of this sampling method requires that sampling units must be taken sequentially until the cumulative number of larvae exceeds stop line values for the number of sample units collected. The mean density can then be estimated as the quotient of the cumulative number of larvae divided by the number of sample units. The larvae stop lines showed that the required sample size increased with the precision level increased. For example, only 11 sample units needed to be inspected to achieve D= 0.25 when mean density was 3.6 larvae per sample unit. However sample size increased dramatically to 67 to achieve precision level of $D = 0.1$. In this study, when $D = 0.25$, densities > 4 larvae per sample unit required < 11 samples, but densities of < 1 larvae required > 32 samples (Fig. 1 and 2).

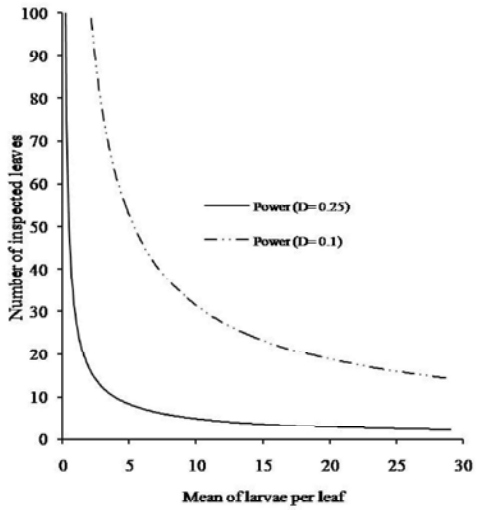

Fig. 1: The required sample size for fixed- precision sequential sampling (D = 0.1 and 0.25) of *Liriomyza sativae* larvae

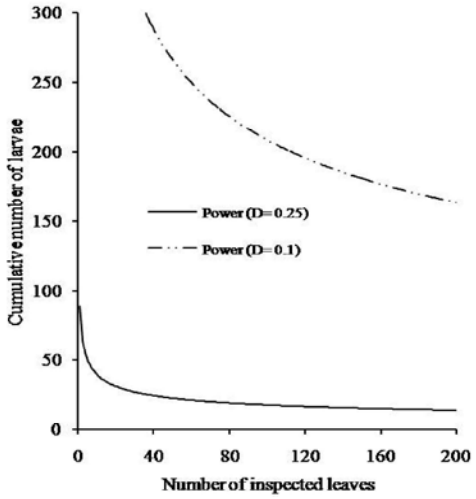

Fig. 2: Sequential sampling stop lines for fixed- precision level (D) of 0.1 and 0.25 for various *Liriomyza sativae* larval densities

In another study, Lee *et al*. [28] stated that when $D = 0.25$, densities > 4 leafmines per sample unit required < 39 samples, but densities of < 1 leafmine required > 67

samples. Parrella and Jones [39] suggested sequential sampling plans using sticky yellow traps with two large and small sizes for trapping mature insects of *L. trifolii* in chrysanthemum greenhouse. They proposed that with a precision of 0.25 only 18% of the traps were needed to be counted. In another study using Taylor index coefficients, Heinz and Chaney [32] designed a sequential sampling plan for *Liriomyza huidobrensis* larvae on celery, which was very precise in estimating decision-making lines regarding the aggregated frequency of larvae and larval channels.

We concluded that the spatial distribution of *L. sativae* larvae in cucumber greenhouses was of aggregated form. However, the distribution was random in some sampling dates, in particular, where the population density was low. Given the advantages of sequential sampling in pest population management, the present study attempted to design some models for sequential sampling of local populations. These findings allow the larvae population mean and other associated parameters to be estimated faster in favor of smaller sample size.

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