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Full Length Research Paper

Estimation of larval density of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in cucumber greenhouses using fixed precision sequential sampling plans

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This study was conducted to develop sequential sampling plans to estimate larval density of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) at three precision levels in cucumber greenhouse. The within- greenhouse spatial patterns of larvae were aggregated. The slopes and intercepts of both Iwao's patchiness regression and Taylor's power law did not differ between years. A fixed-precision level sampling plan was developed using the parameters of Taylor's power law generated from total number of larvae in a cucumber leaf at three precision levels (*D*) of 0.1, 0.25 and 0.28. The resulting sampling plans were tested with sequential bootstrap simulations (n = 500) using 10 independent data sets for validation. Bootstrap simulation within a wide range of densities demonstrated that actual *D*' values at desired *D*= 0.28 averaged < 0.28 in all cases. Even at the lowest density of larvae (0.24 larvae per leaf), the actual mean *D*' was 0.25 at *D*= 0.28. This result shows that the sampling plan developed in this study is effective and reliable for estimating the larval densities in cucumber greenhouses.

Key words: Vegetable leafminer, sequential sampling, spatial distribution, greenhouse cucumber.

INTRODUCTION

Leafminers belonging to the genus *Liriomyza* (Diptera: Agromyzidae) are regarded as pests in many crops due to their damage to leaves (López et al., 2010). *Liriomyza* genus includes about 300 species distributed worldwide with 23 species being considered economically important (Parrella, 1987; Kang et al., 2009). The leafminer 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 (López et al., 2010). In Iran, *L. sativae* was first seen in 2000. This species and *Liriomyza trifolii* Burgess have seriously damaged beans, peas, vegetables and tomatoes in the provinces of Khuzestan, Kerman and Tehran (Askary, 1995; Javadzadeh, 2004). At present, *L. sativae* mixed with *L. trifolii* 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 (López et al., 2010). 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 (Capinera, 2005).

Leafminers have a relatively short life cycle; they are able to complete their development in 21 to 28 days under warm environments such as Florida. In tropical climates, numerous generations occur annually (Capinera, 2001). Leibee (1984), determined growth at a

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constant 25°C, and reported that about 19 days were required from egg deposition to emergence of the adult.

The management of agromyzid leafminers 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 (Murphy and Lasalle, 1999).

Spatial distribution is a behavioral response of the individuals of a species to habitat (Southwood, 1995; Young and Young, 1998). The information of special distribution (i.e., regular, random or aggregated) can determine what sampling program must be carried out, especially sequential sampling (Elliot and Kiechhefer, 1986; Feng et al., 1993).

A successful management of leafminers strongly depends on the development of an appropriate sampling plan (that is, it is easy to be implemented and suitable for rapid decision-making processes). In sampling programs, precision and cost-effectiveness are two most important factors that need to be considered (Pedigo, 1994). For example, compared with fixed-sample size sampling, a fixed-precision sequential sampling can result in a 35 to 50% reduction in sampling effort (Binns, 1994). 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 leafminer densities in cucumber greenhouses.

MATERIALS AND METHODS

The study site

Field experiments were carried out at an experimental greenhouse located in Jiroft (Kerman, Iran) during growing seasons of November to April in from 2007 to 2009. The cucumber *Cucumis sativus* cv. RS189 I SINA F1 (Royal Sluis, Netherlands) was grown under greenhouse on four 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 (Southwood and Henderson, 2000).

Relative variation (RV) was used to compare the efficiency of various sampling methods (Hillhouse and Pitre, 1974). 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

Taylor's power law

Taylor's power law was calculated as follows:

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

Where, *a* and *b* are scaling factors related to sample size and an index of aggregation, respectively (Southwood and Henderson, 2000).

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 differences between years' distribution coefficients were tested by the statistic, t ($t = \frac{b_1 - b_2}{\sqrt{SE_1^2 + SE_2^2}}$) (Feng and

Table 1. Estimated parameters from primary sampling of *Liriomyza sativae* on cucumber during 2007 to 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 Taylor's power law regression analysis.

Growing season	b ± SE	Log[a] ± SE	R^2	F	t	Df
2007-2008	1.174 ± 0.063	0.295 ± 0.03	0.937	344.212**	2.76*	23
2008-2009	1.317 ± 0.074	0.264 ± 0.066	0.94	313.974**	4.28*	20
Overall	1.263 ± 0.051	0.272 ± 0.036	0.934	607.279**	5.15*	44

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

Nowierski, 1992a, b). 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.

Sequential sampling planning

Green's (1970) model was used for designing a sequential sampling plan with the precisions of 0.1, 0.25 and 0.28. The required sample number for estimating mean population was estimated by $n = \frac{ax^{(b-2)}}{D^2}$ and decision lines were estimated by $T_n = \left(\frac{an^{1-b}}{D^2}\right)^{\frac{1}{(2-b)}}$ (Pedigo and Buntin, 1994). 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 (Southwood and Henderson, 2000).

Validation of sampling plans

Actual precision levels obtained from the sequential sampling program at specified levels of precision were evaluated by bootstrap simulation (Efron and Tibshirani, 1986). The simulations were performed on independently collected data sets not used in developing the sampling plan. For this purpose, 10 independent data sets were collected in 2009. The mean densities of these data sets ranged from 0.24 to 27.75 larvae per leaf. The sample size of each data set consisted of 35 leaves.

Re-sampling for Validation of Sampling Plans (RVSP) software developed by Naranjo and Hutchison (1997) was used for bootstrap simulations. The RVSP was used to resample each of 10 data sets with a replacement option until the stop line had been reached. In addition to the initial fixed- precision levels of 0.1, 0.25 and 0.28, a minimum sample size of five was used for all simulations. Resampling was repeated 500 times for each data set, producing the average precision level and the average, minimum and maximum sample size.

RESULTS AND DISCUSSIONS

Sampling program

The results of primary sampling show 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 two growing seasons, respectively. These RVs were very appropriate for the sampling program (Table 1).

Spatial distribution

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 equations 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<

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 ± 0.15	0.972	702.934**	5.02*	20
Overall	1.208 ± 0.03	0. 48 ± 0.274	0.972	1607.386**	6.93*	44

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

0.05; Table 3), implying an aggregated distribution.

Hence, given the high precision of both Taylor's and Iwao's coefficients, the former was used for estimating spatial distribution, and for designing sequential sampling plans, because Taylor index is not changed with environmental variations (Nestel et al., 1995) and not being affected by sample size (Croft et al., 1976). The comparison of annual distribution coefficients using *t*-statistic showed no significant difference (t_{slope} =1.47 and 1.06, $t_{intercept}$ = 1.87 and 1.05, P <0.05 for Taylor's and Iwao's coefficients respectively). Therefore, the annual data was pooled between years, and overall distribution coefficients were calculated (Tables 2 and 3).

Previous studies have been stated an aggregated form for the spatial distribution pattern of *Liriomyza* sp. (Lee et al., 2005). Here, the estimated Taylor index *b* was between 1.17 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 (Burgio et al., 2005), 1.15 and 1.19 for *L. trifolii* channels and larvae on chrysanthemum (Jones and Parrella, 1986),1.16 for *L. sativae* on beans (Hanna et al., 1987), 1.19 for *L. huidobrensis* larvae on celery (Heinz and Chaney, 1995), 1.51 for *L. trifolii* larvae on celery (Beck et al., 1981), and 1.62 for *L. trifolii* leafmines on greenhouse tomatoes (Lee et al., 2005).

In this study, the estimated Iwao index *β* was between 1.15 and 1.24. In another study, the estimated values of this index for leafmines, 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 (Lee et al., 2005), 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 (Burgio et al., 2005).

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 0.28 and 0.25, the number of samples required for estimating the population density of *L. sativae* larvae varied between two to 157 and three to 197 leaves respectively, 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 to 1229 leaves under the same larval densities (Figure 1).

Fixed- precision sequential sampling stop lines were calculated at three levels of precision (Figure 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 nine sample units needed to be inspected to achieve D= 0.28 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, *D*= 0.25, densities > 4 larvae per sample unit required < 11 samples, but densities of < 1 larvae required > 32 samples (Figure 2 and Table 4).

Several sampling programs have been developed for different *Liriomyza* species on greenhouse and field vegetable crops. Musgrave et al. (1975) found that yellow sticky traps could be used for rapid detection of adult *L. trifolii* population fluctuation and Parrella and Jones (1985) suggested sequential sampling plans using yellow sticky 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. Jonson et al. (1980) suggested that the pupal tray survey was a fast and accurate method of estimating pupal density.

Although monitoring of leafminer adult or pupal stages may be accomplished with relatively simple tools, these methods produce either large estimation errors or contain inherent time delays by predicting subsequent rather than present leafminer densities (Trumble and Nakakihara, 1983; Parrella et al., 1989). Moreover, the relationship between adults trapped and larval densities in plants is difficult to elucidate, particularly in commercial greenhouses where applications of pesticides cause adult and larval populations to fluctuate dramatically (Parrella and Jones, 1985). In other studies using Taylor

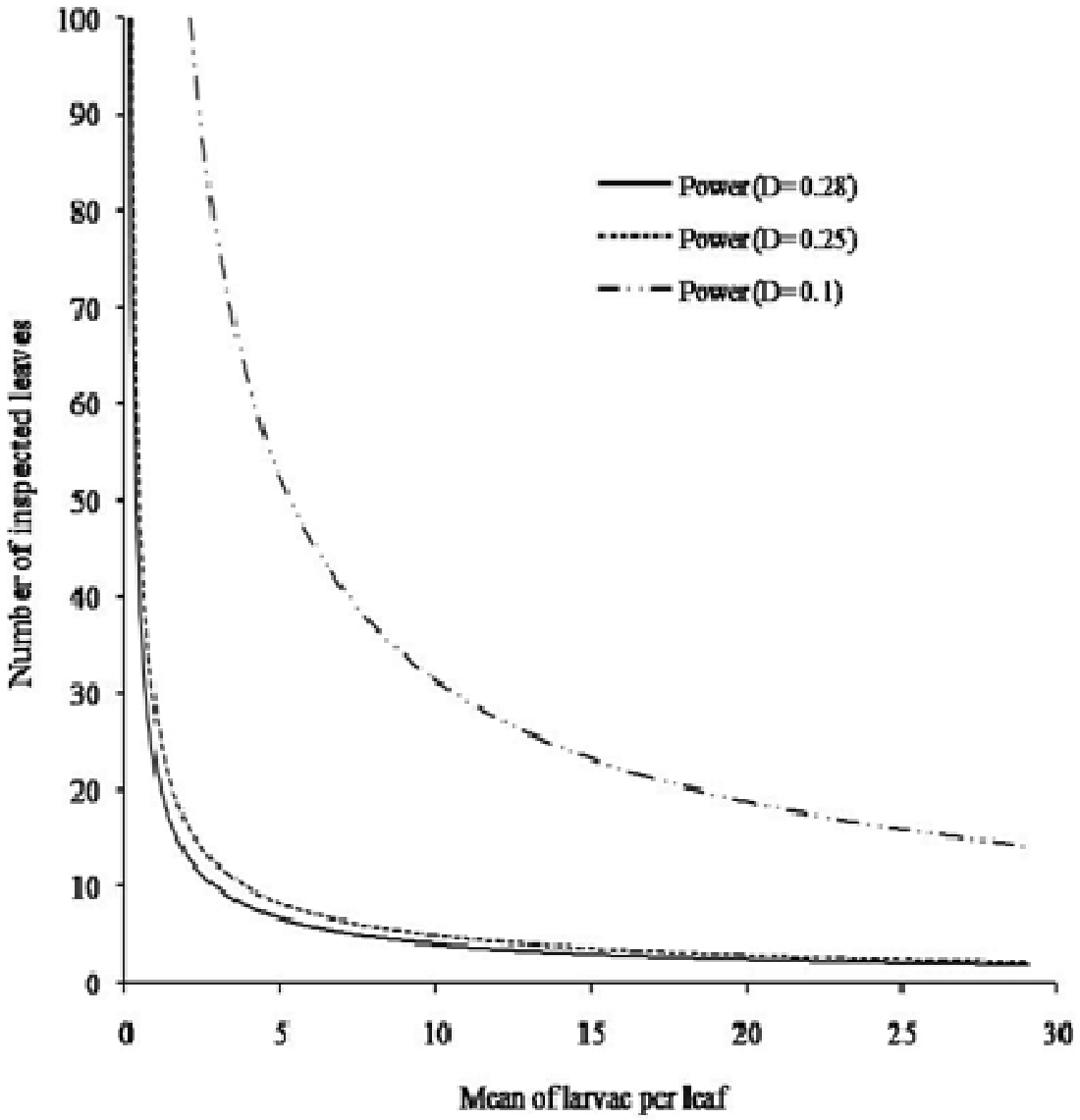

Figure 1. The required sample size for fixed- precision sequential sampling (D = 0.28, 0.25 and 0.1) of *Liriomyza sativae* larvae.

index coefficients, Heinz and Chaney (1995) and Lee et al. (2005) designed sequential sampling plans for *L. huidobrensis* larvae on celery and *L. trifolii* leafmines on tomato respectively, which were very precise in estimating decision-making lines regarding the aggregated frequency of larvae and leafmines.

Counting live larvae has two advantages over yellow sticky trap sampling and pupal tray survey. Firstly, the larval sampling allows assessing the damage easily because the major source of the damage by *Liriomyza* species is the accumulation of leafmines during the growing seasons (Chandler and Gilstrap, 1987). The other is that result of the larval sampling data can be directly incorporated into a control decision-making program. Also the knowledge of population levels of live larvae allows control actions to be based on population levels present, rather than on a calendar type or prophylactic schedule. In addition, use of the larval sampling plan allows evaluation of pesticide efficacy, which may provide a rapid indication of control failure (Lee et al., 2005). Therefore, the larval sampling program is needed to improve timing of control measures, and to facilitate the establishment of economic threshold values.

Validation of sampling plans

Variability in precision level, density estimation and sample size from simulation sampling were used as criteria for evaluating performance of the fixed- precision

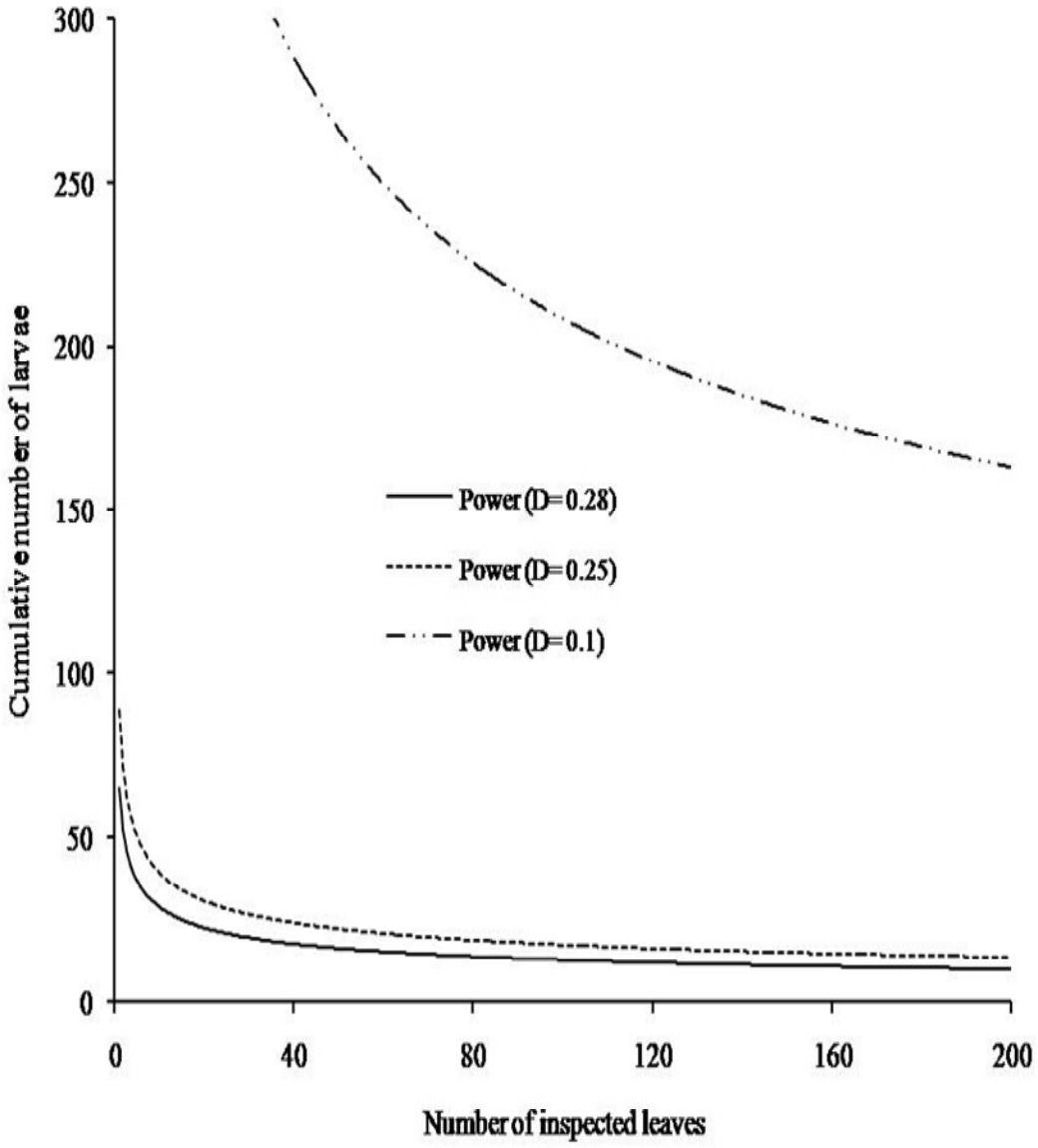

Figure 2. Sequential sampling stop lines for fixed- precision level (D) of 0.1, 0.25 and 0.28 for various *Liriomyza sativae* larval densities.

sequential sampling plan according to Hutchison et al. (1988). A sampling plan is considered reliable only if > 90% of the observed *D*' values are less than the desired *D* (Hutchison, 1994). In nine out of 10 data sets (for *D*= 0.1) and all other data sets (for *D*= 0.25 and 0.28), the observed *D'* values were less than or equal to the desired *D* values, indicating that the plan was reliable (Table 4).The estimated means (*m'*) also did not differ significantly with the actual means (*m*) for all data sets on which simulation were performed and at all levels of precisions. The simulation runs also provided the information on variability in the required sample size (Table 4). The required sample size was more variable for low densities (m< 0.5) than for intermediate (0.5< m< 6) and high densities (m> 6).

In pest management programs, reduced cost may be worth a loss in precision as long as precision is sufficient to make correct decisions. The simulation results indicate that the relaxed desired precision level of *D*= 0.28 was acceptable and practicable because the averaged observed *D'*= 0.245 was sufficient for pest management purpose (Table 4). The similar results have been observed for *L. trifolii* by Lee et al. (2005) recommending that the precision of 0.3 would be sufficient in sampling programs, and other arthropods, and these results illustrate the need for validation process (O´Rourke and Hutchison, 2003). Thus based on the simulation results, the sampling plan with *D*= 0.28 is recommended for *L.*

Table 4. Statistics for a 500 simulation runs for a fixed- precision sequential sampling plan with desired precision levels (D) of 0.1, 0.25 and 0.28 on ten independent data sets collected in 2009.

Data set[a]		Statistics for 500 simulation runs				
		Sample size				
Series	**m ± SE**	**m'**	**Mean**	**Max**	**Min**	**Average *D'***
Desired *D* = 0.1						
1	0.24 ± 0.087	0.25	480	577	200	0.09
2	0.3 ± 0.096	0.3	428	513	200	0.1
3	0.43 ± 0.11	0.43	324	398	200	0.1
4	0.67 ± 0.18	0.65	237	282	198	0.09
5	0.88 ± 0.16	0.86	195	240	159	0.09
6	1.44 ± 0.25	1.47	132	168	100	0.1
7	3.64 ± 0.49	3.67	67	81	51	0.1
8	6.02 ± 0.55	6.04	46	57	38	0.09
9	8.76 ± 0.69	8.66	36	44	30	0.08
10	27.35 ± 2.87	27.81	15	20	12	0.11
Average	4.97 ± 2.64	5.01	196	238	118.8	0.095
Desired *D* = 0.25						
1	0.24 ± 0.087	0.26	80	121	42	0.23
2	0.3 ± 0.096	0.31	70	112	37	0.24
3	0.43 ± 0.11	0.44	54	95	33	0.25
4	0.67 ± 0.18	0.68	39	60	25	0.2
5	0.88 ± 0.16	0.89	32	53	21	0.23
6	1.44 ± 0.25	1.52	22	37	13	0.25
7	3.64 ± 0.49	3.74	11	18	7	0.24
8	6.02 ± 0.55	6.26	8	13	5	0.2
9	8.76 ± 0.69	8.98	6	9	5	0.19
10	27.35 ± 2.87	27.64	5	5	5	0.21
Average	4.97 ± 2.64	5.07	32.7	52.3	19.3	0.224
Desired *D* = 0.28						
1	0.24 ± 0.087	0.26	63	107	38	0.25
2	0.3 ± 0.096	0.31	56	109	31	0.27
3	0.43 ± 0.11	0.44	43	79	24	0.27
4	0.67 ± 0.18	0.67	31	46	16	0.23
5	0.88 ± 0.16	0.91	25	43	15	0.25
6	1.44 ± 0.25	1.54	18	29	9	0.28
7	3.64 ± 0.49	3.76	9	16	5	0.26
8	6.02 ± 0.55	6.23	6	10	5	0.22
9	8.76 ± 0.69	8.59	5	7	5	0.21
10	27.35 ± 2.87	27.67	5	5	5	0.21
Average	4.97 ± 2.64	5.04	26.1	45.1	15.3	0.245

[a] Each data set contained 35 observations.

sativae management applications.

In conclusion, our study indicates that the spatial distribution of *L. sativae* larvae in cucumber greenhouses was of aggregated form and the fixed precision sampling scheme developed using Green's method was acceptable for estimating larval densities in commercial cucumber greenhouses. Therefore, the sampling strategies provided here can be used to obtain a rapid estimate of larval densities with minimal effort. In addition, the knowledge of density level of larvae would provide the solid basis for optimal decision- making in IPM programs for *L. sativae*.

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