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Fixed precision sequential sampling plans for leaf mines of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in cucumber greenhouses

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ABSTRACT

This study was conducted to develop sequential sampling plans to estimate leaf mine density by *Liriomyza sativae* (Blanchard) at three fixed-precision levels in cucumber greenhouse. The within- greenhouse spatial patterns of leafmines were aggregated. The slopes and intercepts of 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 leafmines in a cucumber leaf at three precision levels (*D*) of 0.1, 0.25 and 0.29. 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*= 0.29 averaged less than or equal to 0.29 in all cases. Even at the lowest density of leaf mine (0.27 mine per leaf), the actual mean *D*' was 0.28 at *D*= 0.29. This result shows that the sampling plan developed in this study is effective and reliable for estimating the mine densities in cucumber greenhouses.

Keywords: Vegetable leaf miner, leaf mine, sequential sampling, spatial distribution, resampling simulation, greenhouse cucumber

INTRODUCTION

Leafminers belonging to the genus *Liriomyza* (Diptera: Agromyzidae) are regarded as pests in many crops due to their damage to leaves (Lopez *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 (Lopez *et al.* 2010). In Iran, *L. sativae* was first seen in 2000. This species and *L. trifolii* Burgess have seriously damaged beans, peas, vegetables and tomatoes in the provinces of Khuzestan, Kerman and Tehran (Askary 1995, Kalantar hormozy *et al.* 2000, Javadzadeh 2004). 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 (Lopez *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 21-28 days under warm environments such as Florida. In tropical climates, numerous generations occur annually (Capinera 2001). Leibee (1984), 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 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 (Young and Young 1998, Southwood 1995). The information of spatial distribution (i.e., regular, random or aggregated) can determine what sampling program must be carried out, especially sequential sampling (Feng *et al.* 1993, Elliot and Kiechhefer 1986).

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 (Pedigo 1994). For example, compared with fixed-sample size sampling, a fixed-precision sequential sampling can result in a 35-50% reduction in sampling effort (Binns 1994). The development of a sequential sampling scheme with a fixed statistical precision, therefore, may be useful for estimating leaf mine density by *L. sativae* 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 leafmines developed by *L. sativae* larvae, and to develop and evaluate a fixed-precision sequential sampling for estimating leaf mine 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 program

One single leaf of a cucumber plant was selected as a sample unit. Then, it was inspected *in situ* to record the total number of leafmines of *L. sativae* per leaf. 40 Cucumber leaves were randomly sampled and counted for mine density of *L. sativae* once a week during morning.

Sampling plan development

Dispersion indices of leafmines were calculated using Taylor's power law as following:

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

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

The values of F and P acquired from regression equations were used to test whether the Taylor's coefficient (b) was 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 coefficients and their standard deviations in regression equations, respectively.

Since Taylor's coefficients were estimated by two-year data, the difference of distribution coefficients between years, were tested by the statistic t ($t = \frac{b_1 - b_2}{\sqrt{SE_1^2 + SE_2^2}}$) (Feng and Nowierski 1992a, b). Here, b_1 (and SE_1) and b_2 (and SE_2) are the Taylor's coefficients (and their standard errors) 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.

Coefficients from the Taylor's power law regression were used to develop fixed-precision sequential sampling plan. The sampling stop line was calculated by following formula (Green 1970):

$$\log T_n = \frac{\log\left(D^2 / a\right)}{b - 2} + \frac{b - 1}{b - 2} \log n \quad (1)$$

Where T_n is the cumulative number of leafmines over n samples, D is the fixed level of precision, and a and b are from the Taylor's regression. Precision levels were set between 0.1 and 0.29 because estimates of population density within 10% and 25% of the mean are considered adequate for ecological studies and pest management decisions respectively (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, ten independent data sets were collected in 2009. The mean densities of these data sets ranged from 0.27 to 31.83 mines per leaf. The sample size of each data set consisted of 40 leaves.

Resampling 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 ten 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 0.29, a minimum sample size of 3 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 DISCUSSION

Spatial distribution

Mean numbers of leaf mines per leaf ranged from 0.26 to 12.48 in 2008 and from 0.07 to 37.4 in 2009. Taylor's equations for the growing seasons were obtained as $\log S^2 = 0.249 + 1.181 \log m$ (F_{26} = 277.405, $P< 0.05$; Table 1) and $\log S^2 = 0.259 + 1.219 \log m$ (F_{23} = 1641.347, $P< 0.05$), both with a great degree of fit (> 0.90). In addition, the coefficient b was significantly greater than 1 (2007-2008: $t_{26} = 2.55$, $P< 0.05$; 2008-2009: $t_{23} = 7.3$, $P< 0.05$; Table 1), implying an aggregated distribution.

Table 1: 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	1.181 ± 0.071	0.249 ± 0.034	0.917	277.405^{**}	2.55^{*}	26
2008-2009	1.219 ± 0.03	0.259 ± 0.028	0.985	1641.347^{**}	7.3^{*}	23
Overall	1.217 ± 0.036	0.251 ± 0.022	0.97	1629.611^{**}	7.23^{*}	50

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

The comparison of annual distribution coefficients using *t*-statistic showed no significant difference (t_{slope} =0.31 and $t_{intercept}$ = 0.75, P <0.05). Therefore, the annual data was pooled between years, and overall distribution coefficients were calculated (Tables 1).

Previous studies have been stated an aggregated form for the spatial distribution pattern of *Liriomyza* spp. (Zenjun 1997; Yan 1998; Hammad and Nemer 2000; Lee *et al.* 2005). Here, the estimated Taylor index *b* was between1.181 and 1.219. 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* mines 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* mines on greenhouse tomatoes (Lee *et al.* 2005).

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 (Zenjun 1997, Yan 1998, Burgio *et al.* 2005).

Sequential sampling

Fixed- precision sequential sampling stop lines were calculated using equation 1 at three levels of precision (Fig. 1).

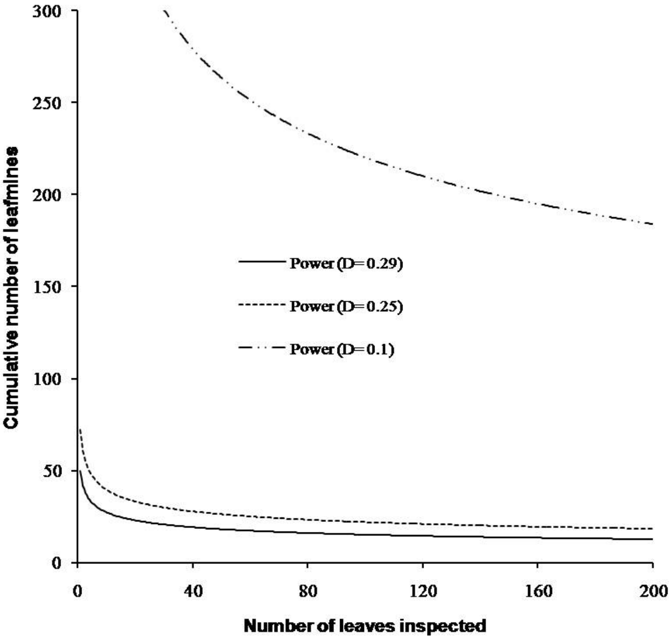

Fig. 1: Sequential sampling stop lines for fixed- precision level (D) of 0.1, 0.25 and 0.29 for various *Liriomyza sativae* leaf mine densities.

Utilization of this sampling method requires that sampling units must be taken sequentially until the cumulative number of mines 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 leafmines divided by the number of sample units. The leaf mines stop lines showed that the required sample size increased with the precision level increased. For example, only 9 sample units needed to be inspected to achieve D= 0.29 when mean density was 3.42 leafmines per sample unit. However sample size increased dramatically to 71 to achieve precision level of *D*= 0.1(Table 2).

Table 2: Statistics for a 500 simulation runs for a fixed- precision sequential sampling plan with desired precision levels (D) of 0.1, 0.25 and 0.29 on ten independent data sets collected in 2009.

		Statistics for 500 simulation runs				
Data set[a]			Sample size			
Series	m ± SE	m'	mean	max	min	Average *D'*
Desired *D* = 0.1						
1	0.27 ± 0.11	0.28	516	650	200	0.10
2	0.54 ± 0.16	0.52	316	383	200	0.09
3	0.76 ± 0.17	0.75	237	281	196	0.08
4	1.33 ± 0.28	1.34	151	187	117	0.10
5	1.74 ± 0.42	1.73	123	166	89	0.10
6	2.27 ± 0.36	2.32	97	118	81	0.09
7	3.42 ± 0.41	3.48	71	85	61	0.10
8	6.5 ± 1.04	6.69	42	54	31	0.09
9	10.93 ± 1.43	11.4	28	37	23	0.09
10	31.83 ± 3.85	31.76	13	17	10	0.11
Average	5.86 ± 2.96	6.03	159.4	197.8	100.8	0.095
Desired *D* = 0.25						
1	0.27 ± 0.11	0.29	85	143	46	0.24
2	0.54 ± 0.16	0.54	52	80	29	0.22
3	0.76 ± 0.17	0.77	39	68	24	0.20
4	1.33 ± 0.28	1.4	25	46	13	0.24
5	1.74 ± 0.42	1.82	20	33	10	0.26
6	2.27 ± 0.36	2.43	16	25	10	0.21
7	3.42 ± 0.41	3.54	12	17	8	0.20
8	6.5 ± 1.04	6.94	7	13	4	0.24
9	10.93 ± 1.43	11.63	5	8	3	0.19
10	31.83 ± 3.85	31.74	3	4	3	0.21
Average	5.86 ± 2.96	6.11	26.4	43.7	26.4	0.221
Desired *D* = 0.29						
1	0.27 ± 0.11	0.3	63	104	28	0.28
2	0.54 ± 0.16	0.55	38	62	23	0.26
3	0.76 ± 0.17	0.78	29	47	16	0.24
4	1.33 ± 0.28	1.45	19	39	9	0.28
5	1.74 ± 0.42	1.88	15	28	8	0.28
6	2.27 ± 0.36	2.43	12	22	7	0.24
7	3.42 ± 0.41	3.52	9	15	6	0.21
8	6.5 ± 1.04	7.02	6	12	3	0.29
9	10.93 ± 1.43	11.84	4	7	3	0.21
10	31.83 ± 3.85	31.28	3	3	3	0.20
Average	5.86 ± 2.96	6.1	19.8	33.9	10.6	0.249

[a] Each data set contained 40 observations.

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 another study using Taylor index coefficients, Heinz and Chaney (1995) designed a sequential sampling plan for *L. huidobrensis* larvae on celery, which was very precise in estimating decision-making lines regarding the aggregated frequency of larvae and larval channels.

Counting mines has two advantages over yellow sticky trap sampling and pupal tray survey. Firstly, the leafmine 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 leafmine sampling data can be directly incorporated into a control decision-making program. Therefore, the leafmine sampling program is needed to improve timing of control measures, and to facilitate the establishment of economic threshold values (Lee *et al.* 2005).

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 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 9 out of 10 data sets, the observed *D'* values were less than or equal to the desired *D* values, indicating that the plan was reliable (Table 2).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 2). The required sample size was more variable for low densities (m< 2) than for intermediate (2< m< 6.5) and high densities (m> 6.5).

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 indicated that the relaxed desired precision level of *D*= 0.29 was acceptable and practicable because the averaged observed *D*'= 0.249 was sufficient for pest management purpose (Table 2). 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*= 0.29 is recommended for *L. sativae* management applications.

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

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