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Research Article

Fixed-precision sequential sampling to estimate the population density of *Heliothis viriplaca* in bean fields of Shush District, Southwest Iran

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Abstact

The marbled clover *Heliothis viriplaca* is an important pest of beans and other legumes in many regions of the world. The development of precise and cost-benefit sampling methods plays a crucial role in the successful implementation of integrated pest management programs. The spatial distribution of *H. viriplaca* was investigated in a half-hectare research field in Amele-Seif County, Shush District, southwest Iran. Based on the data obtained, a fixed-precision sequential sampling plan was developed to estimate the population of this pest. The spatial distribution of these larvae on bean plants was evaluated using the Taylor and Iwao models. The spatial distribution of this pest on bean plants was found to be uniform, with the Taylor index providing a better fit for the spatial distribution data of larvae on the host plants. Therefore, Green's model was used to develop a fixed-precision sequential sampling plan. To obtain the desired precision levels 0.1 or 0.25, the optimal sample sizes varied from 2.6 to 576 or 0.4 to 90 plants depending on the pest population density. Calculating the sampling stopping lines indicated that sampling for the pest should continue until the cumulative number of larvae on bean plants reaches 0.015 larvae when aiming for precision levels of 0.1 or 0.25.

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Introduction

Beans belong to the genus *Phaseolus*, consisting

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of approximately 220 different species. Approximately 20 species of this genus are cultivated for their green pods or dry seeds. Bean cultivation is most common in tropical regions worldwide. It is grown on all five continents and is considered an important plant-based protein source in many developing countries, containing approximately 22 to 25% protein and 56-57% carbohydrates (Biddle 2017). The global cultivated area for this plant exceeds 3.27 million hectares, with an average yield of approximately 660 kilograms per hectare. In Iran, the cultivated area for this crop is approximately 440,000 hectares, with an average yield of 1500 kilograms per hectare (Sarafraz & Shaaban 2020).

Many economically significant arthropod pests attack bean plants every year in different regions of Iran and cause major cause yield losses (Khanjani 2005). Among the three species of the genus *Heliothis* found in bean fields within Iran, *H. viriplaca* Hüfnagel (Lepidoptera: Noctuidae) is the most prevalent. In fact, approximately 94% of the active population of this genus is attributed to this particular species (Khanjani 2005). Additionally, 92% of the damage occurring during the larval stage is caused by the last instar. Therefore, any control measures should be implemented prior to the larvae reaching the fourth instar (Khanjani 2007). In recent years, *H. viriplaca* has emerged as a significant pest of soybean, cotton, beets, peas, beans, etc. Severe damage can lead to yield reductions ranging from 40% to 90% (Cui *et al.* 2018). Similarly, in the west Asia, *H. viriplaca* poses a major threat to chickpea and bean crops, causing substantial damage. In certain years, the yield of crops has been observed to decline by approximately 90% (Kahrarian 2009, 2012; Kahrarian *et al.* 2010). During their early stages, the larvae primarily consume the leaf parenchyma and reproductive organs of chickpeas or beans, including flowers and buds. As the pods develop, medium and large larvae enter them and commence feeding on the seeds (Khanjani 2005; Ashtari *et al.* 2022).

To design and implement an effective integrated pest management (IPM) program, accurate knowledge of pest population characteristics, including seasonal population dynamics, spatial distributions and precise and appropriate sampling methods of the pest population, is crucial (Pedigo 2002; Yarahmadi & Rajabpour, 2015; Pedigo *et al.* 2021). Therefore, the design and implementation of a suitable sampling program establish the foundation needed for making informed decisions in the execution of various IPM strategies and tactics. The success of these programs heavily relies on the accuracy and credibility of the sampling plan for estimating pest population status in fields or orchards. Accuracy and cost-effectiveness are two essential factors in developing a successful sampling program (Pedigo 2002; Dent & Binks 2020; Pedigo *et al.* 2021). Fixed-precision sequential sampling is an efficient sampling strategy that minimizes the needed number of samples to achieve the desired accuracy. Therefore, sequential sampling plans play a vital role in increasing the efficiency of sampling efforts. It is estimated that sequential sampling plans can save 35% to 50% in costs and time consumption compared to fixed-size sampling plans while increasing sampling efficiency (with maintained desired accuracy) (Binns 1994).

Although, sequential sampling plans for same noctuid pests were developed on some other important agricultural crops especially on corn (Hoffmann *et al*. 1991; O'Rourke & Hutchison 2003; Serra & Trumper 2006), but no published study has yet been conducted to investigate on the development of a sequential sampling program for *Heliothis* spp. in bean fields or on any other agricultural, vegetable, or horticultural crop worldwide. Considering the importance of this information, the study aimed to develop an appropriate fixed-precision sequential sampling of *H. viriplaca* larvae on bean plants.

Materials and Methods

Experimental design

This experiment was conducted in a half-hectare field in Amale-Seif County, Shush District, northern Khuzestan Province (32.199989N, 48.224155E) during two growing season (September 2022 to April 2023). The seeds of the bean (cultivar Mohali) were used for cultivation. The cultivation was performed in a row and hill system, with a distance of 60 centimeters between rows and 20 centimeters between plants. Weed control was carried out manually using hoes, and no pesticides (including insecticides or herbicides) were applied in the experimental field during the study period. Irrigation and soil management were performed according to the recommendations of the local agricultural department.

Sampling

Samplings were conducted weekly. All samplings were performed in the morning (from 7 to 11 am). On each sampling date, a pattern of X-shaped movement was followed on two diagonals of the field, and every 10 steps, a plant was randomly selected, and the number of bean pods with pest damage on each plant was counted and recorded separately.

Determining spatial distribution

Two indices, Taylor's power law and Iwao's patchiness indices, were used to study the spatial distribution of *H. viriplaca* larvae on bean plants.

Taylor's law is expressed as:

$$S^2 = a\bar{X}^b$$

Where S^2 is the popultion variance, $\bar{X}$ the population mean, and the parameter *a* is a constant value, which specific to each insect species. This parameter is defined as the sampling factor. The parameter *b* represents the aggregation index of the insect population. To calculate the values of *a* and *b*, a regression equation was established between the logarithm of variance ($\log S^2$) as the dependent variable and the logarithm of the mean ($\log \bar{X}$) as the independent variable, as described in the following equation. In this equation, the slope of the regression line (*b*) indicates the type of spatial distribution of the insect population. Values greater than, equal to, and less than one represent aggregated, random, and uniform distributions, respectively (Taylor 1984).

$$\log S^2 = \log a + b \log \bar{X}$$

Iwao's patchiness

To calculate this index, a linear regression between the mean of crowding (X^*) and the mean population of the insect ($\bar{X}$) was established, as described in the equation below (Iwao, 1975):

$$X^* = \alpha + \beta\bar{X}$$

In this equation, α represents the intrinsic properties of the species, and β represents the type of spatial distribution of the insect population in its habitat. The values of X^* can be calculated based on the mean and variance of the population using the following equation:

$$X^* = \bar{X} + \left(\frac{S^2}{\bar{X}} - 1\right)$$

Considering that a significant difference in *b* in Taylor's index or β in Iwao's index with a value of one serves as a criterion for determining the type of spatial distribution of the pest, the *t* test was used to test the difference between *b* and one (Afshari & Dastranj 2010; Rajabpour 2022). The calculation of the *t*-statistic based on the slope of the regression line for Taylor's and Iwao's equations can be performed using the following equation:

$$t = \frac{(slope - 1)}{(SE\ slope)}$$

Sequential sampling plan

In this study, considering that Taylor's index had a better capability to fit the data of the spatial distribution of *H. viriplaca* larvae on the bean plants, Green's model was used to develop fixed-precision sequential sampling at desired precision levels of 25 and 10%. In Green's model, the minimum sample size needed to achieve the desired precision is determined by the following equation.

$$n = \frac{a\bar{X}^{b-2}}{D^2}$$

Where *n* is the needed sample size for estimating the population at the desired precision level, and *a* and *b* are the intercept and slope in Taylor's regression equation, respectively. The sampling stop lines (or decision lines) in Green's model, which indicate the stopping time of sampling, can be estimated using the following equation:

$$T_n \geq \left(\frac{an^{1-b}}{D^2}\right)^{\frac{1}{(2-b)}}$$

Where T_n is the cumulative larval number of the pest in n sample size, and the other parameters are as described in the previous equations (Naranjo & Hutchison 1997).

Results

The results of the analysis of spatial distribution data of *H. viriplaca* larvae on the bean plants, based on the Taylor and Iwao regression models, are shown in Figure 1. The regression analysis results of both models indicated that the Taylor power law had a higher coefficient than Iwao's patchiness, suggesting a better fit for the spatial distribution data of larvae of this pest on bean plants. Considering the regression line equation of the Taylor and Iwao models and the fact that the slope of the regression line for both models was less than 1, it can be concluded that the spatial distribution type of pea aphid larvae on bean plants is uniform.

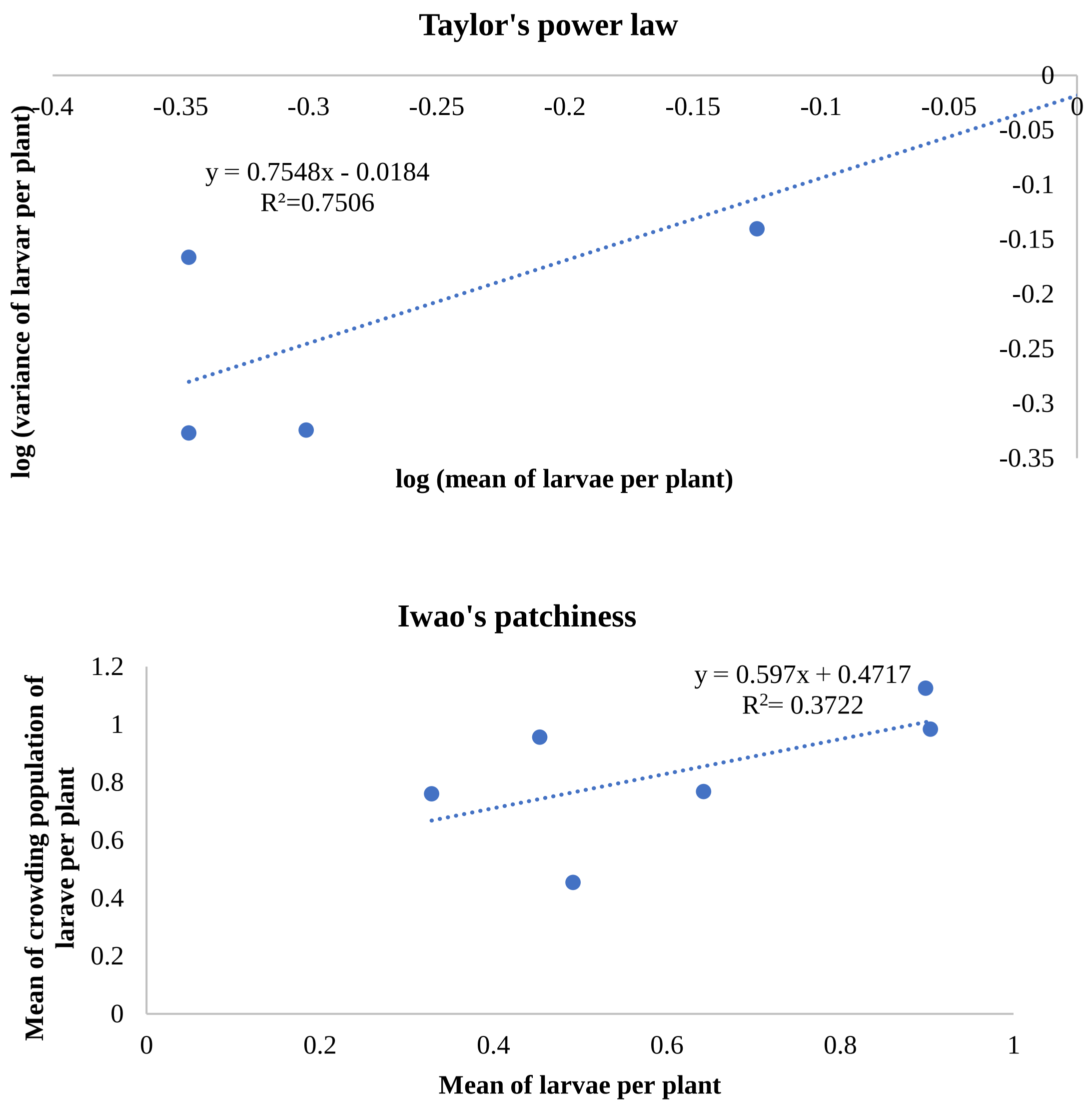

Figure 1. Regressions according to Taylor's power law and Iwao's patchiness models to assess spatial distribution of *Heliothis viriplaca* larvae on bean plants.

In Figure 2, the optimal sample size needed for estimating the population of *H. viriplaca* larvae on the bean plants is shown for precision levels of 0.25 (for integrated pest management purposes) and 0.10 (for research purposes). Based on the results obtained from Green's model, the optimal sample size varies depending on the pest density, ranging from 576 to 2.6 plants for a desired precision level of 0.10 or 90 to 4.0 plants for a needed precision level of 0.25.

The results obtained indicated that for the 0.25 precision level, sampling for the pest larvae should continue until the cumulative number of larvae reaches 0.0015 per bean plant. This stop line was also applicable for the 0.10 precision level. The findings of this study were consistent with Green's model (1970), which stated that the stopping line for a uniform distribution increases with the increasing number of sampled plants, with a uniform slope or an upward trend.

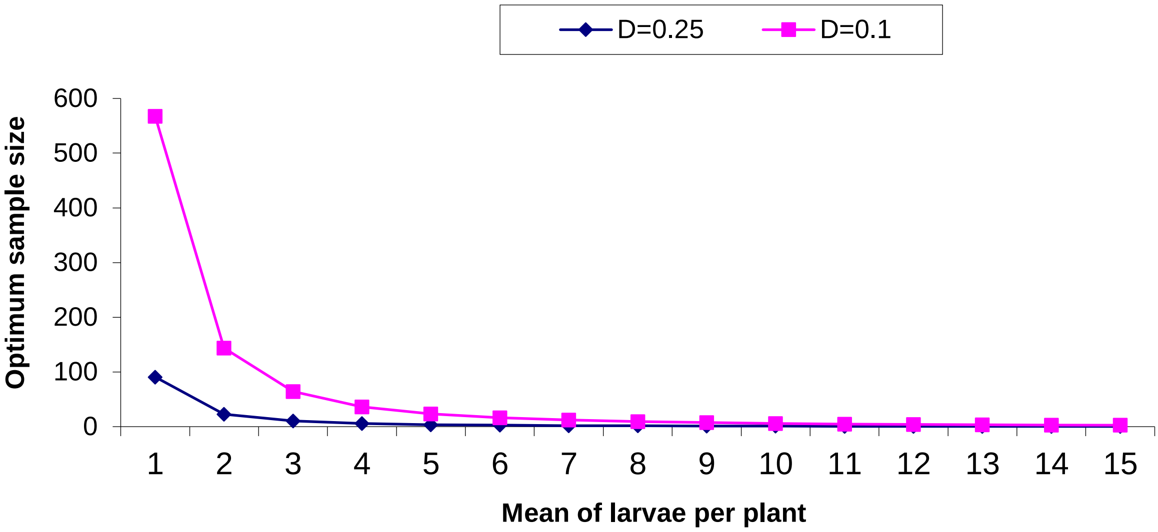

Figure 2. The optimal sample size required for accurately estimating the larval density of *Heliothis viriplaca* on bean plants based on the Green's model at precision levels of 0.25 or 0.1.

Discussion

Our findings indicated that the spatial distribution of *H. viriplaca* larvae on bean plants is uniform. Using abundant data of trapped males in pheromone traps in various locations over three seasons, Taylor's power law indicated a strong cumulative spatial aggregation of *H. armigera* Hübner and *H. punctigera* Wallengren moths, as significant pests of cotton and other agricultural crops in Australia, both temporally and spatially (Fitt *et al.* 1989). In another study on the spatial distribution of *Helicoverpa* spp. species in tomato fields using Taylor's power law, it was shown that the distribution of their eggs is cumulative. The slope of the Taylor regression line in this study was 1.59, which differed from the current study (Dawson *et al.* 2006).

However, for *H. assulta* Guenee larvae on hot chili peppers, it was demonstrated that the spatial distribution, based on damaged pepper fruit data by *H. assulta*, follows a random pattern according to the Taylor power law (Baek *et al.* 2009). Similar results were obtained for *H. armigera* larvae on chickpea pods in terms of random distribution based on Taylor's spatial distribution model (Patnaik & Senapati 2002), both of which differed from the current study. The spatial distribution of any species depends on its behavior (Taylor 1984). One of the factors that affects the behavior and consequently the spatial distribution of a pest species is its host plant type (Mohammadi *et al*. 2015; Dinarvand *et al*. 2020). Therefore, the difference in the slope or intercept values calculated in the regression line equation of the Taylor and Iwao models in the present study compared to the aforementioned previous studies may be due to differences in the host plant of this pest larvae and the behavioral effects of different hosts. Similar to the current study, previous studies also found that the Taylor model provided a better fit for the spatial distribution data of black bean aphids on other plant hosts.

The obtained findings indicated that the needed sample size is always higher at the 0.10 precision level than at the 0.25 precision level, and as pest density increases, the needed sample size decreases significantly. In other words, the optimal sample size is a function of the desired precision level and the larval density in the field. The reason for the need for larger sample sizes at lower densities is the relationship between the mean and variance of pest density, as expressed by the slope of the Taylor regression (Rashidi *et al.* 2020).

Because there is no other published study on developing a sequential sampling plan for monitoring the population of *Heliothis* spp., it is not possible to compare the developed sequential sampling plan in this study with other research conducted on larvae of this pest on other plants or other species of lepidopteran pests on different hosts or beans because all fixed-precision sequential sampling models (such as Green's or Kuno's model) depend on the spatial distribution parameters of the pest species. Spatial distribution is one of the ecological characteristics of species; therefore, the parameters vary based on different pest species, and it is obvious that the developed sampling plan in this case is unique and cannot be generalized to other pest species. Furthermore, many factors related to the host plant, environmental conditions, various competitions, etc., can influence the spatial distribution parameters and consequently affect the developed sequential sampling model (Taylor 1984). For example, it is domenstrated that the host plant characteristics can influence the biological characteristics of pests (Shohabi & Rajabpour 2017) demonstrated that even changing the potato variety, considering different morphological conditions and resistance factors, has a significant impact on the spatial distribution and consequently the needed sample size or stopping lines of sequential sampling based on sequential sampling models (e.g., the Green model). Similarly, Kafshani *et al.* (2018) showed that the spatial distribution parameters and consequently the sampling plan for a pest on different citrus species (Thomson orange and Satsuma mandarin) exhibit significant differences. likewise, the spatial distribution parameters and sequential sampling (optimal sample size or stopping lines) for two noctuid species larvae, *Spodoptera exigua* Hübner and *Sesamia cretica* Led., on a specific host plant (corn) showed significant differences (Dinarvand *et al.* 2020). Moreover, studies conducted on fixed precision sequential sampling for *H. zea* Boddie larvae on corn demonstrated that the stopping lines for sampling this pest are 0.10 and 0.80 per plant (Calero-Toledo *et al.* 2008), which is completely different from our obtained results.

In conclusion, The spatial distribution of *H. viriplaca* larvae on bean plants, based on Taylor's and Iwao's models, showed a uniform pattern, and the Taylor power law indicated a better fit for the spatial distribution data of these larvae on the host plan. The optimal number of needed sample sizes varies depending on pest density, ranging from 2.6 to 576 bushes for a desired precision level of 0.1 or 0.4 to 90 plants for a desired precision level of 0.25. Calculation of stop sampling lines showed that the sampling should continue until the cumulative number of pest larvae on bean plants reaches 0.015. The results of this study could play an important role in developing an effective IPM program for *H. viriplaca* in bean fields.

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پژوهش‌های کاربردی در گیاه‌پزشکی ۱۴ (۱): ۲۱-۱۳ (۱۴۰۴)
مقاله پژوهشی

برنامه نمونه‌برداری دنباله‌ای با دقت ثابت برای تخمین تراکم جمعیت *Heliothis viriplaca* در مزارع لوبیای شهرستان شوش (جنوب غرب ایران)

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چکیده

کرم پیله‌خوار *Heliothis viriplaca* یک آفت مهم لوبیا و سایر حبوبات در بسیاری از مناطق دنیا به شمار می‌رود. توسعه روش‌های نمونه‌برداری دقیق و مقرون به صرفه، نقش مهمی در اجرای موفقیت آمیز برنامه‌های مدیریت تلفیقی آفات ایفا می‌کند. در این پژوهش پراکنش فضایی لاروهای *H. viriplaca* در یک مزرعه تحقیقاتی نیم هکتاری در شهرستان شوش در جنوب غربی ایران مورد بررسی قرار گرفت. بر اساس داده‌های به دست آمده، یک برنامه نمونه‌برداری دنباله‌ای با دقت ثابت برای برآورد جمعیت این آفت تهیه شد. توزیع فضایی لاروهای این آفت بر روی گیاهان لوبیا با استفاده از مدل‌های تیلور و ایوائو ارزیابی شد. توزیع فضایی این آفت روی گیاهان لوبیا از نوع یکنواخت بود و شاخص تیلور برازش بهتری برای داده‌های توزیع فضایی لاروهای این آفت روی گیاه میزبان داشت. بنابراین، از مدل گرین برای تهیه یک برنامه نمونه‌برداری دنباله‌ای با دقت ثابت این آفت، استفاده شد. تعداد بهینه نمونه‌های مورد نیاز بسته به تراکم آفت از۲/۶ تا ۵۷۶ بوته برای سطح دقت مطلوب ۰/۱ یا ۰/۴ تا ۹۰ بوته برای سطح دقت مورد نیاز ۰/۲۵ متغیر بود. محاسبه خطوط توقف نمونه‌برداری برای سطح دقت مطلوب ۰/۱ و ۰/۲۵ نشان داد که نمونه‌برداری برای آفت باید ادامه یابد تا زمانی که تعداد تجمعی لارو در بوته‌های لوبیا به ۰/۰۱۵ لارو برسد، ادامه یابد.

کلمات کلیدی: اندازه نمونه، برآورد جمعیت، پراکنش فضایی، مدل گرین، مدیریت تلفیقی آفات