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Article

Spatial distribution and sampling plan for *Tetranychus urticae* (Acari: Tetranychidae) in bean crops

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ABSTRACT
The two-spotted spider mite, *Tetranychus urticae* Koch, is one of the most destructive bean pests in the central part of Iran. Spider mites feeding on bean leaves cause physiological changes in the plant, which coupled with favorable environmental conditions can lead to increased mite infestations. Significant yield loss can occur in the absence of pest monitoring and timely management. Monitoring for its occurrence and the population density is usually done by visual inspection and consequently demands much effort and time. Hence the objectives of this study were 1) to describe the sampling distribution pattern of this mite and 2) to develop a fixed-precision sequential sampling plan for density estimation. Population estimates were made by registering the number of all stages on 150 leaves of bean plants from mid to the end of July of 2021. Taylor's power law (TPL) was used to analyze the spatial distribution of the pest (r^2 = 0.849). Aggregation Indexes (b = 1.239) were significantly greater than one, indicating the aggregation of *T. urticae*. We used TPL parameters to develop a sequential sampling plan at precision levels of 0.10 and 0.25 using Green's method. Fixed-precision sequential sampling plan could represent a suitable method for sampling *T. urticae* population in bean fields in the Varamin area, with the practical advantage of lower cost and time consumption than standard sampling plans. The use of this plan outside the Varamin area can be applicable. In conclusion, the sequential sampling plans determined in this study for mites can be incorporated into integrated pest management programs in bean crops by making precise and quick decisions.

KEY WORDS: Fixed sample size, *Phaseolus vulgaris*, precision, sequential sampling, two-spotted spider mite.

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an agriculturally important legume crop worldwide. Common bean is widely cultivated in different parts of Iran and occupies more than 120,000 ha (Rahmani *et al.* 2011).

Mites of the family Tetranychidae (commonly known as spider mites) are important pests in agricultural and forestry ecosystems and can be found on many field crops, fruit trees, vegetables,

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and ornamental plants. Many spider mites naturally inhabit ephemeral and patchily distributed resources such as weeds. The most notorious and important tetranychid mite is the globally distributed two-spotted spider mite, *Tetranychus urticae* Koch (Hamedi 2022). It is a polyphagous pest, which occurs on a wide range of host plants such as common bean crops in Iran. So far, *T. urticae* has been reported to feed on about 4000 host plant species worldwide (Migeon and Dorkeld 2021). It can decrease the photosynthetic rate of common bean plants by 14–20%, causing a reduction in yield by about 50% (Shaabani *et al.* 2021).

A survey of bean cropping systems has shown that chemical control is the main control method used by producers and involves the use of broad-spectrum miticides, mainly chlorfenapyr (Polaris ® 24% SC), abamectin (Vertimec®, 0.5ml/l) and floramite (Bifenazite®24% SC) without any sampling plan (Farazmand *et al.* 2019). The chemical acaricides used to control Tetranychidae are characterized by a large variety of chemical structures and modes of action which were reviewed by Attia *et al.* (2013) and Dekeyser (2005). A more reliable approach to treatment decisions requires estimating mite densities to determine when they exceed the action threshold. A sampling procedure designed to support mite management decisions must balance the reliability of a population estimate against the cost of achieving that estimate (Margolies *et al.* 1984). However, the use of pesticides cannot be eliminated in a short period in perennial crops because other control methods might not be able to maintain the spider mite populations below the economically acceptable level on their own; it can be reduced by a lot of strategies such as the successful utilization of a precise sampling plan (Khodayari and Hamedi 2021). In other words, adopting a sampling protocol with an acceptable level of precision and logistical feasibility may reduce unnecessary insecticide applications (De Araujo *et al.* 2020). Despite the importance of *T. urticae* as a major pest in bean crops, a sampling plan for use in IPM programs for this species has yet to be developed for beans. Control methods must be adopted to prevent two-spotted spider mite populations from reaching densities capable of causing economic loss. In this context, sampling plans are important for pest control decision-making (Pedigo and Buntin 1993).

Sampling plans must be simple, representative, accurate, quick to implement, and low-cost (Gusmão *et al.* 2005; Lopes *et al.* 2019). In addition, they must enable decision-making in fields of different sizes and at any phenological stage of the crop (Lopes *et al.* 2019).

A key component of an IPM program is regular monitoring of the pest population. Therefore, the first objective of this study was to analyze the spatial distribution of this mite on bean plants and the second was to develop two sampling plans: a sampling plan with fixed levels of precision for estimating *T. urticae* populations and a sequential sampling plan to classify the pest.

MATERIALS AND METHODS

Field counts

The data for this study was obtained from samples of *T. urticae* populations taken in 13, ~1-ha fields of common bean (*P. vulgaris*) the central part of Pishva, in Varamin vicinity, Tehran province, Iran. These fields were sampled six times (two-day intervals) from mid to the end of July.

Sampling

Each field was divided into five equal areas from one edge to the other. Thirty plants were chosen randomly by traveling in each sampling area and one leaf taken from the mid-height of a plant (i.e., a total of 150 leaves) was selected from each field. Each field was sampled six times from mid to end of July to record the total number of mite population per leaf. The leaves were individually placed in plastic bags and refrigerated at 4 °C. The number of *T. urticae* (eggs, immature stages, and adults) was determined on each leaf using a stereo-microscope (OLYMPUS, SZH-ILLB) in the laboratory.

Spatial distribution

An initial step in developing a sampling plan is to determine the spatial distribution of the target pest within the field (Krebs 1999; Southwood and Henderson 2009). For this, means and variances of counts of *T. urticae* were calculated for each sampling occasion. Indexes of dispersion were calculated using Taylor's power law $s^2 = am^b$, which describes the variance-to-mean relationship (Taylor 1961). The Taylor's power law parameters were obtained through a linear regression of the natural logarithms of the variances on the natural logarithms of the means of each sampling occasion, adjusting them in a linear regression to obtain the linear equation of the model, $ln(s^2) = ln(a) + b\ ln(m)$, where the parameter a is a scaling factor related to sample size (Southwood and Henderson 2009) and the slope b is an index of aggregation which indicates a uniform ($b < 1$), random ($b = 1$) and aggregated ($b > 1$) dispersion of population. Student tests were used to determine if the slopes b of the regression lines were significantly different from the unit. The precision of b values, according to Downing (1986), was estimated by calculating the relationship SE_b/b, where values < 0.2 indicate its robustness.

Sample size

Taylor's power law coefficients mentioned above can be used in determining optimum sample size for simple random sampling (Iwao and Kuno 1971; Karandinos 1976; Ruesink 1980). Using precision defined as D, which is expressed as a percentage standard error of the mean. Precision levels of 0.10 and 0.25 were chosen for this study. The optimum sample size can be expressed by Green's (1970) formula:

$$n = {am^{b-2}}/{D^2}$$

where a and b are Taylor's power law coefficients, m *T. urticae* density, and D is the precision.

Development of sampling plans

Sampling plans were developed based on fixed precision level stop lines using Taylor's parameters. Substitution of the variance as expressed by Taylor's power law into the sequential estimation method, developed by Green (1970), allows the calculation of critical stop lines using the equation:

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

where T_n is the cumulative number of *T. urticae* in a sample of n leaves and defines the sequential sampling stop line.

RESULTS AND DISCUSSION

According to this study, the mean density of the population of *T. urticae* per leaf ranged from 84.233 ± 1.998 to 0.793 ± 0.093. The highest population of *T. urticae* per leaf was counted only in ~10% of fields. Since the variance is greater than the mean of the observed data, a clumped pattern was reasonably suspected (Fig. 1). Taylor's power law described the spatial distributions of the *T. urticae* (Table 1). Aggregation was confirmed by parameter b of Taylor's power law (Table 1). As such values were > 1, they indicate that *T. urticae* follows an aggregate distribution. The calculation of the SE_b/b (0.048) showed that a value < 0.2, which supports the precision in the determination of b (Table 1). This, according to Downing (1986), is considered strong evidence to support the procedure. Our data confirm the fit of the mean and variance to Taylor's power law (Table 1). Similar results have been found in other studies on different bean cultivars (Ahmadi *et al.* 2005; Mehrkhou *et al*. 2008; Sedaratian *et al*. 2008; Pakyari 2012; Mohiseni and Kushki 2016) and for different crops such as tart cherry (Jones 1990), almond (Wilson *et al.* 1984), carambola (Shih and Wang 1996), strawberry (Greco *et al.* 1999), clementine (Martinez-Ferrer *et al.* 2006), rose (So 1991; Bidarnamani *et al.* 2015), apple (Slone and Croft 1998) and peanut (Margolies *et al.* 1984). The characterization of spatial distribution of *T. urticae* in beans has provided crucial information for the development of an effective sampling plan which will ultimately improve the monitoring of this pest and assist in effective pest management targeting this economically important pest. Within a bean field, the mite population is characteristically spatially patchy, both at small (Ahmadi *et al.* 2005, Mehrkhou *et al*. 2008; Sedaratian *et al*. 2008; Pakyari 2012; Mohiseni and Kushki 2016) and field scale (Mohiseni and Kushki 2016 and this study). Knowledge, regarding such spatial distributions and how they develop through space and time, is important because such information may be used to optimize insecticide usage through selective spraying (Farazmand *et al.* 2019). Additionally, spatial pattern mediates interactions between mites and their natural enemies and therefore impacts of biological control (Pakyari 2012).

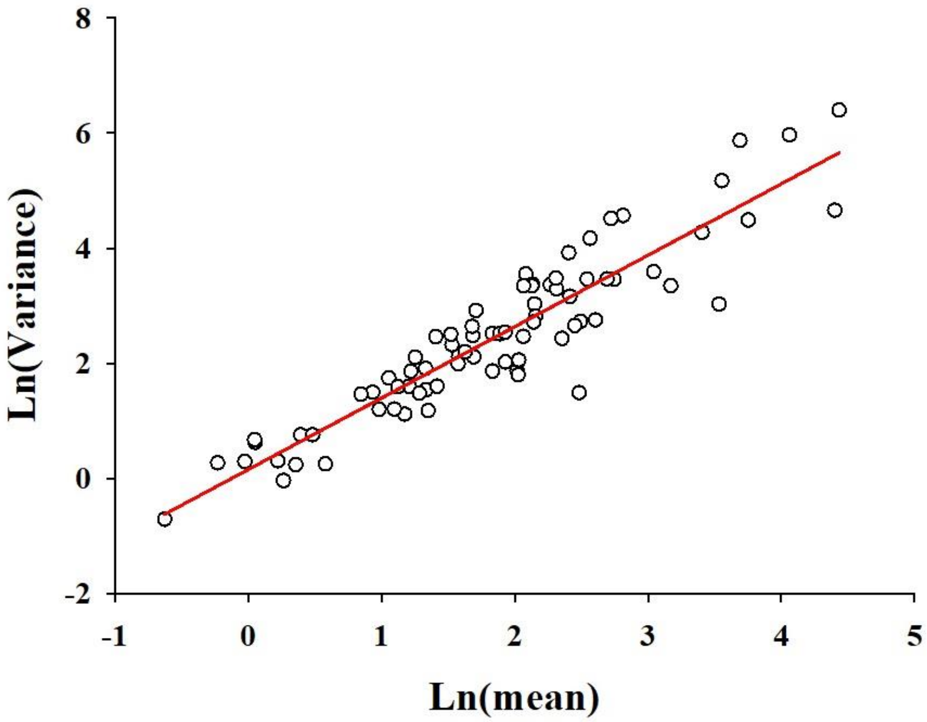

Figure 1. Relationship between variance and mean density (all stages combined per leaf) of *T. urticae* samples collected from bean fields near Varamin vicinity, Tehran province, Iran. The red lines are the best-fitting lines of Taylor's power law.

Table 1. Parameters of Taylor's power law regressions for leaf samples of *T. urticae* and test for differences of slopes from units taken from bean fields near Varamin vicinity, Tehran province, Iran.

Number of data series	Parameter estimation						Test for slope≠1	
	Intercept ± S.E.	Slope ± S.E.	*adj* r^2	*F*	d.f.	*P*	t = (slope-1)/S.E.$_{slope}$	*P*
78	1.171 ± 0.127	1.239 ± 0.059	0.849	435.88	77	< 0.0001	4.037	0.001

The optimum sample sizes for *T. urticae* at two different levels of precision are shown in Fig. 2.

Sample size requirements for the desired precision level of 0.10 and 0.25 declined rapidly as mean density increased (Fig. 2). The maximum mean sample size for densities of one mite per leaf at a precision of 0.10 was 118 and the maximum mean sample size for densities of one mite at a precision of 0.25 was 19. As the mean density of *T. urticae* increased to 5 and 15 (mite/leaf), the mean sample size decreased at both 0.10 (35 and 15 samples respectively) and 0.25 (6 and 3 samples respectively) precision levels.

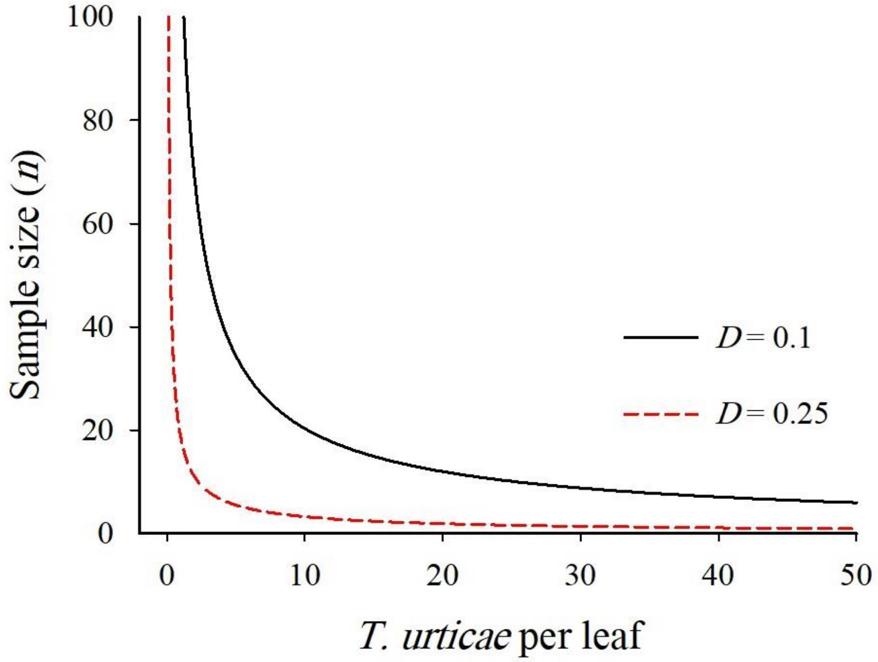

Figure 2. Sample sizes required to achieve a given precision level of 10 and 25% at different mean densities of *T. urticae* per leaf.

Mohiseni and Kushki (2016) indicated that the optimum sample size for *T. urticae* for varying numbers of leaves sampled per bean plant (2, 4, and 6 leaves) was 146, 102, and 86 bean plants per field for the precision level of 0.25. This was greater than our result and achieving their result is very time-consuming, laborious, and impractical (Hutchison 1994; Henderson 2021). Hutchison *et al.* (1988) discussed the over-estimation of sample size that they suggest in these cases should be used in lower levels of precision. In our study, the average sample size needed to classify the density of mites rarely exceeded 20 even at densities near 5 mites per leaf, however, at a density lower than 3 mites per leaf, the sample size could exceed 100.

According to our study, for a field study, it is difficult to use a fixed sample size, because counting the number of mites on an individual leaf is the most time-consuming aspect of surveying. Therefore, a sequential count plan is more desirable, where an estimate of the population mean has a fixed precision (Allen *et al.* 1972). A sequential count plan would be suitable for the monitoring program, as it is necessary to obtain a relatively good estimate of the mean.

The sequential sampling plan ($D = 0.1$ and $D = 0.25$) is presented in Figure 3. Dotted lines show three different population levels that are drawn on the diagram. The sequential count plan can be used in the field in the following manner: Random samples of one leaf are taken and the number of *T. urticae* is noted. The cumulative number of *T. urticae* is plotted against the sample number and when a stop line is crossed the sampling stops. The population density, as a mean number of *T. urticae* per sample unit, can then be calculated, and compared with the action threshold to make a spray decision. Sampling for a precision level of 10% is not feasible in an IPM situation. Because for estimating a mean density of *T. urticae* per leaf at the 10% level of precision, more samples are necessary than at the 25% level. In low-population levels, there will be many leaves without any mites then sample sizes will be unreasonable. Counting the number of mites on an individual leaf is the most time-consuming and if a stop line has not been crossed after 20 to 40 samples (depending on the desired precision level), the sampler should stop anyway, as population levels are probably too low to result above the economic threshold. Sampling for population levels around the economic threshold can be more easily accomplished by using a sequential decision plan.

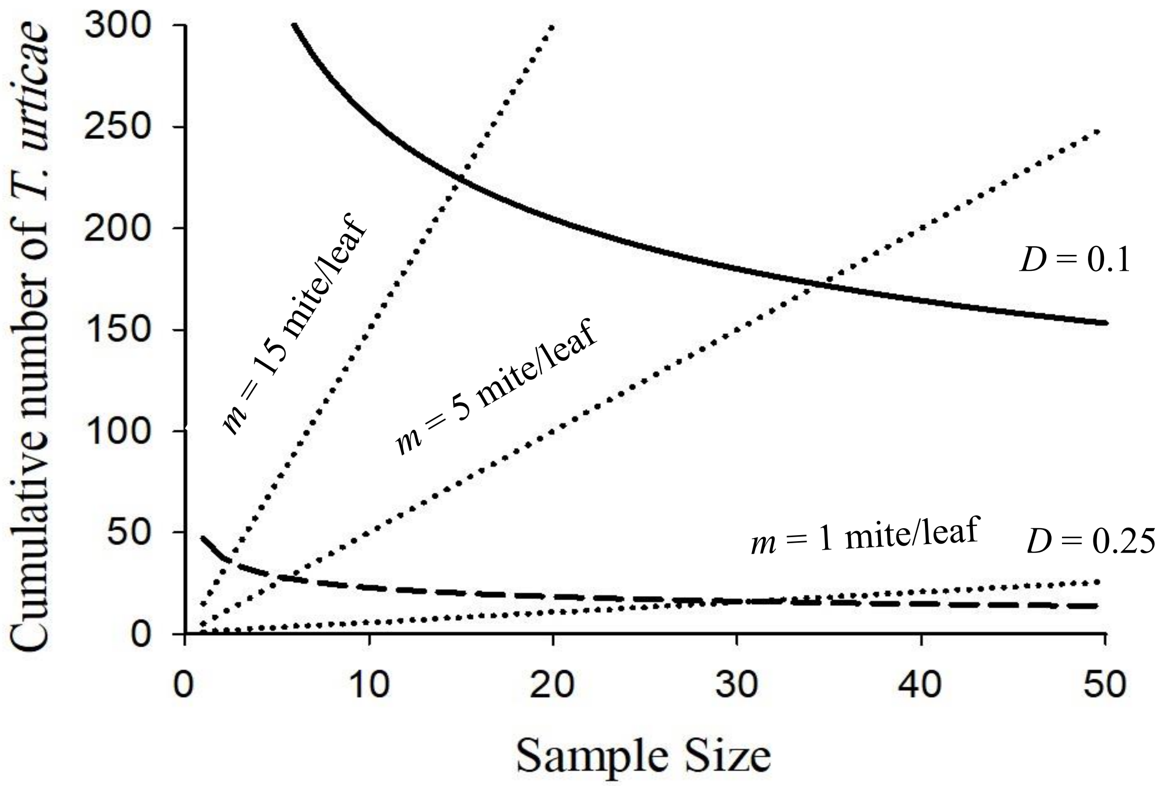

Figure 3. Critical stop lines for a sequential count plan for *T. urticae*. For a precision level of 10 and 25%.

One major obstacle to implement IPM programs in bean crops is the lack of sampling plans. The sequential sampling plans, provide decisions for any pest density (Young and Young 2013). Furthermore, sequential plans provide precise, quick, and low-cost decisions (Pedigo and Buntin 1993). Decision-making sampling systems for *T. urticae* presented here offer value for bean growers. This study generated sequential sampling plans of *T. urticae* eggs, immature stages, and adults during the bean cultivation to provide more accurate and fast decisions according to a grower's control methods. These decision-making systems help to exhibit a favorable cost-benefit ratio for growers (De Paulo Arcanjo *et al.* 2021). An important fact of the sampling plans, determined in this study is the considered methods used in bean crops in pesticide applications. This makes mite control decisions economical, efficient, and adapted to the management system used by the farmer. Furthermore, these sequential sampling plans can make accurate decisions at any pest density with a minimum sample and could save sampling time. Bean sampling plans should improve sampling efficiency and be simpler to implement. On the other hand, the sampling procedure must be compatible with sampling programs currently in use for other bean pests. Although the mite sampling procedure presented in this research was developed to be efficient, further studies are necessary to determine the compatibility with existing pest sampling programs.

In conclusion, the sequential sampling plans, determined in this study for mites can be incorporated into integrated pest management programs in bean crops by making precise and quick decisions.

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پراکنش پهنه‌ای و طرح نمونه‌برداری از *Tetranychus urticae* (Acari: Tetranychidae) در کشتزار لوبیا

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چکیده

کنهٔ تارتن دولکه‌ای، *Tetranychus urticae* Koch یکی از مخرب‌ترین آفات لوبیا در مناطق مرکزی ایران است. این کنه‌ها با تغذیه از محتویات سلولی برگ‌های لوبیا باعث تغییرات فیزیولوژیکی در گیاه می‌شوند که همراه با شرایط محیطی مساعد می‌تواند منجر به افزایش آلودگی کنه‌ها شود. در صورت عدم پایش آفات و مدیریت به موقع، کاهش بسیار عملکرد می تواند رخ دهد. نظارت بر وقوع آن و تراکم جمعیت معمولاً با بازرسی چشمی انجام می‌شود و در نتیجه نیازمند تلاش و زمان زیادی است. از این رو اهداف این مطالعه ۱) توصیف الگوی توزیع نمونه‌برداری از این کنه و ۲) ایجاد یک طرح نمونه‌برداری پیاپی با دقت ثابت برای تخمین تراکم بود. تخمین جمعیت با ثبت تعداد تمام مراحل روی ۱۵۰ برگ بوته لوبیا از اواسط تا پایان تیر ماه ۱۴۰۰ انجام شد. برای تجزیه و تحلیل پراکنش پهنه‌ای آفت از قانون توان تیلور (TPL) استفاده شد (r^2 = ۰/۸۴۹). شاخص های تجمع (b = ۱/۲۳۹) به طور معنی داری بیشتر از یک بود که نشان دهنده تجمع *T. urticae* است. از آماره‌های TPL برای توسعه یک طرح نمونه‌برداری پیاپی در سطوح دقت ۰/۱۰ و ۰/۲۵ با استفاده از روش گرین استفاده شد. طرح نمونه‌برداری پیاپی با دقت ثابت می‌تواند روشی مناسب برای نمونه‌برداری از جمعیت *T. urticae* در کشتزارهای لوبیا در منطقه ورامین با مزیت عملی هزینه و زمان کمتر نسبت به طرح‌های نمونه‌برداری استاندارد باشد. استفاده از این طرح در خارج از محدودهٔ ورامین قابل اجرا است. در نتیجه، طرح‌های نمونه‌برداری متوالی تعیین شده در این مطالعه برای کنه‌ها را می‌توان با تصمیم‌گیری دقیق و سریع در برنامه‌های مدیریت تلفیقی آفات در کشتزارهای لوبیا گنجاند.

واژگان کلیدی: اندازهٔ نمونه ثابت؛ لوبیا، تخمین، نمونه‌برداری دنباله‌دار، کنهٔ تارتن دولکه‌ای.

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