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Research

Ecology and Behavior

Spatial distribution and fixed-precision sequential sampling plan for the cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae), on Chinese hibiscus in Southwestern Iran

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The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), has emerged as a key pest of ornamental plants, yet effective monitoring tools for its management remain limited. This study investigated the spatial distribution and developed fixed-precision sequential sampling plans for *P. solenopsis* populations on Chinese hibiscus (*Hibiscus rosa-sinensis* L.) under urban landscape conditions in Khuzestan province, southwest Iran. Spatial distribution analyses using Taylor's power law and Iwao's patchiness regression revealed significant aggregation across developmental stages, with nymphs exhibiting the highest degree of clumping and adults showing weaker aggregation due to greater dispersal ability. Based on these aggregation parameters, sequential sampling plans were generated for nymphs, adults, and combined populations at 3 precision levels ($D = 0.25$, 0.15, and 0.10). Optimum sample size requirements were density-dependent and consistently higher for nymphs than for adults. Validation through resampling confirmed that achieved precision closely matched target levels, with mean sample sizes for combined stages (nymphs and adults) ranging from 47 ($D = 0.25$) to 294 ($D = 0.10$). The $D = 0.25$ plan provided robust accuracy while substantially reducing sampling effort, making it most suitable for practical pest management. By contrast, the $D = 0.10$ plan is recommended for research requiring high precision. These findings provide the first validated sequential sampling framework for *P. solenopsis* on hibiscus, offering an efficient and reliable basis for integrated pest management in urban green spaces.

Keywords: Taylor's power law, Iwao's patchiness regression, mealybug, hibiscus, IPM

Introduction

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), is an invasive, highly polyphagous pest species native to the Americas that has achieved rapid global dispersal, establishing itself across Asia, Africa, and the Middle East (Hodgson et al. 2008, Abbas et al. 2009, Arif et al. 2009, Nagrare et al., 2009; Fand and Suroshe 2015, Spodek et al. 2018). Its feeding damage—including chlorosis, leaf curling, stunting, and copious honeydew production that fosters sooty mold development—causes significant aesthetic and physiological harm to host plants (Arif et al. 2009). In Iran, *P. solenopsis* has emerged as a serious agricultural and ornamental pest, particularly in the southern provinces of Khuzestan, Bushehr, and Hormozgan, where persistently warm climatic conditions facilitate year-round reproduction and population growth (Bagheri and Moghaddam 2010, Mossadegh et al. 2015, Boushi et al. 2020).

While extensive research has addressed the ecology, damage, and management of *P. solenopsis* in agricultural systems such as cotton and vegetables (Jhala et al. 2008, Nagrare et al. 2009, Abbas et al. 2010, Dhawan et al. 2010, Vennila et al. 2010, Wang et al. 2010, Ghada 2021, Waqas et al. 2021, Refaei et al. 2024), its population dynamics on ornamental plants remain critically understudied. This knowledge gap is concerning given that ornamental hosts like Chinese hibiscus (*Hibiscus rosa-sinensis*) provide a highly favorable microhabitat, offering a dense canopy and shelter conducive to mealybug development. Sustained infestations in urban landscapes not only reduce the plants' ornamental value but also act as persistent

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reservoirs, potentially triggering outbreaks in adjacent agricultural areas (Boushi et al. 2020).

Effective management of *P. solenopsis*, consistent with integrated pest management (IPM) principles, is fundamentally dependent on efficient and accurate monitoring. Conventional fixed-sample-size plans are often inefficient for pests with aggregated spatial distributions, frequently leading to oversampling in low-density areas and undersampling where populations are dense, thus compromising decision-making (Binns 1994, Buntin 1994). An efficient and economical sampling strategy is therefore essential for establishing a reliable IPM program (Pedigo 2002). Developing such a plan requires a precise understanding of the pest's spatial distribution pattern (Taylor 1984).

Sequential sampling, particularly fixed-precision sequential sampling (FPSS), offers a robust and flexible alternative by dynamically adjusting the sampling effort in response to real-time population density estimates, thereby optimizing resource allocation (Binns 1994). This method utilizes parameters derived from spatial distribution models, primarily Taylor's power law (TPL) and Iwao's patchiness (IP) regression, to design sampling schemes—Green's model for TPL-based data and Kuno's model for IP-based data (Green 1970, Hutchison et al. 1988, Naranjo and Hutchison 1997). FPSS has been successfully deployed for monitoring various sap-sucking pests, including other mealybug species (Martínez-Ferrer et al. 2006, Beltrà et al. 2013), aphids (Elliott and Kieckhefer 1987, Feng and Nowierski 1992, Ramezani et al. 2016, Zarei-Sarchogha et al. 2019), and whiteflies (Naranjo and Flint 1994), demonstrating reductions in monitoring time and costs by 35% to 50% while maintaining statistical reliability (Binns 1994, Kogan and Herzog 2012).

Despite its proven utility, a sequential sampling plan tailored for *P. solenopsis* on hibiscus is unavailable. Furthermore, stage-specific precision estimates for nymphal and adult populations in urban ornamental habitats are lacking, hindering the development of rapid and cost-effective monitoring protocols needed for IPM programs in urban Iran.

To address key knowledge gaps, this study pursued two primary objectives: (i) to characterize the spatial distribution patterns of different developmental stages (nymphs and adults) of *P. solenopsis* on *Hibiscus rosa-sinensis* using TPL and IP regression, and (ii) to develop stage-specific, FPSS plans at multiple precision levels to facilitate informed and cost-effective management decisions in urban landscapes.

Materials and Methods

Study Site and Sampling

The study was conducted during 2023 and 2024 in urban green spaces of two regions in Khuzestan Province, southwestern Iran (31°18′N, 48°41′E). The area has a hot semihumid climate (Köppen BWh), favorable for year-round development of *P. solenopsis*. Weekly sampling was conducted on 15 randomly selected *H. rosa-sinensis* plants in each of 2 regions (total $N = 30$). To standardize the sampling unit and minimize potential biases associated with vertical and directional canopy variation, shoots were collected from the middle canopy layer across the four cardinal directions per plant. All developmental stages of *P. solenopsis* were counted on a single shoot from each of the 4 strata per plant. These counts were averaged to obtain a mean mealybug density per plant, which served as the experimental unit for subsequent statistical analyses.

Spatial Distribution Analysis

Spatial patterns of nymphs and adults were analyzed using TPL (Taylor 1984) and IP Regression (Iwao 1968).

TPL relates variance (s^2) to mean density ($\dot{x}$), $S^2 = a\bar{x}^b \Rightarrow \log S^2 = \log a + b\log(\bar{x})$, where $b > 1$ indicates aggregation, $b = 1$ randomness, and $b < 1$ uniformity.

IP relates mean crowding (m^*) to mean density, $m^* = \alpha + \beta m$, $m^* = m + \frac{s^2}{m-1}$, where $\beta > 1$ indicates aggregation. Departures of slopes (b, β) from 1 were tested by 1-sample t-tests ($\alpha = 0.05$). Since the Taylor and Iwao coefficients were estimated using pooled data from both years and regions, the homogeneity of the dispersion coefficients between the two regions was verified prior to pooling. This was done using a t-test (Sokal and Rohlf 1987): $t = \frac{b1 - b2}{\sqrt{SE1^2 + SE2^2}}$

with degrees of freedom $= (n_1 + n_2 - 2)$, where "b_1" and "b_2" are the regression coefficients (from either TPL or IP) for regions 1 and 2, and "SE_1" and "SE_2" are their respective standard errors. A nonsignificant difference ($P > 0.05$) confirmed that the data from the two regions could be integrated for a unified analysis.

FPSS Plan

FPSS plans were developed using Green's model (Green 1970), based on TPL parameters. Plans were generated for precision levels of $D = 0.10$, 0.15, and 0.25. The stop line (Tn) was calculated as $Tn = \left(\frac{an^{1-b}}{D^2}\right)^{1/(2-b)}$, and average sample number (ASN) function, which estimates the mean number of samples required to estimate population density at a fixed precision level across a range of densities, was calculated as: $n = \frac{a\bar{X}^{(b-2)}}{D^2}$

Separate plans were produced for nymphs, adults, and total populations. Analyses were performed in Excel.

Sequential Sampling Plan Validation

Validation was conducted using resampling for validation of sampling plans (RVSP) (Naranjo and Hutchison 1997). Ten datasets representing a range of densities from low to high were randomly selected from a pool of 48 collected over 2 years (2023 and 2024). Each dataset was resampled 1,000 times without replacement to assess accuracy and reliability of the sequential sampling plans.

Results

Spatial Distribution of P. solenopsis

The spatial distribution analysis revealed that *P. solenopsis* populations on Chinese hibiscus were aggregated across all developmental stages (Table 1). TPL provided highly significant regressions between mean and variance of insect counts, indicating strong clumping. For nymphs, the slope (b) was 1.876 ± 0.12, significantly greater than unity ($t = 7.31$; df = 18; $P < 0.001$), with a high coefficient of determination ($R^2 = 0.965$).

Table 1. Regression statistics for spatial distribution of *Phenacoccus solenopsis* populations on Chinese hibiscus based on Taylor's power law and Iwao's patchiness regression

Life stage	Model	Slope (±SE)	R^2	*t*-Value	*P*-value	Distribution type
Nymphs	Taylor's power Law	$b = 1.8768 \pm 0.12$	0.965	7.31	<0.001	Aggregated
Adults	Taylor's power Law	$b = 1.7563 \pm 0.09$	0.961	8.40	<0.001	Aggregated
Combined	Taylor's power Law	$b = 1.8177 \pm 0.11$	0.966	7.43	<0.001	Aggregated
Nymphs	Iwao's regression	$\beta = 3.3828 \pm 0.25$	0.933	9.52	<0.001	Aggregated
Adults	Iwao's regression	$\beta = 0.9746 \pm 0.08$	0.970	–0.32	0.756	Random
Combined	Iwao's regression	$\beta = 3.3432 \pm 0.21$	0.955	8.92	<0.001	Aggregated

Adults also exhibited an aggregated distribution ($b = 1.756 \pm 0.09$; $t = 8.40$; $P < 0.001$; $R^2 = 0.960$), though the aggregation intensity was somewhat weaker than in nymphs. When all developmental stages were combined, aggregation remained strong ($b = 1.817 \pm 0.11$; $t = 7.43$; $P < 0.001$; $R^2 = 0.966$).

IP regression corroborated these results (Table 1). Nymphs displayed a high slope value ($\beta = 3.382 \pm 0.25$; $t = 9.52$; $P < 0.001$; $R^2 = 0.933$) and a positive intercept ($\alpha = 13.392$), suggesting the presence of a basic colony unit and strong gregariousness of immature stages. By contrast, adults exhibited a slope ($\beta = 0.974 \pm 0.08$) not significantly different from 1 ($t = -0.32$; $P = 0.756$), reflecting a more random or uniform tendency that may be explained by greater dispersal ability of mature individuals. Analyses using both TPL and IP regression confirmed that *P. solenopsis* populations exhibit aggregated spatial distributions. The degree of aggregation varied by developmental stage, with the highest aggregation observed in the nymphal stage and the lowest in adults (adults displayed a random dispersion pattern according to IP regression). TPL provided a superior model fit, as evidenced by higher coefficients of determination (R^2) compared to Iwao's regression (Table 1), and was therefore selected as the basis for developing the sequential sampling plans. Additionally, a test for homogeneity of regression slopes between regions revealed no significant difference ($t = 1.653$, df = 45, $P > 0.05$), indicating that the spatial aggregation patterns were consistent across study sites.

Sequential Sampling Plans

Based on the aggregation parameters derived from TPL, sequential sampling plans were established for *P. solenopsis* in nymph, adult, and combined populations at three precision levels ($D = 0.25$, 0.15, and 0.10). For nymphs, the required sample size was consistently higher than for the combined stages. At $D = 0.25$, the optimum sample size ranged from 6 to 10 plants across densities of 150 to 0.92 nymphs per shoot. Increasing precision substantially raised sampling effort: at $D = 0.15$, 15 to 30 plants were needed, while at $D = 0.10$, between 30 and 70 plants were required depending on density (Fig. 1). A similar pattern was observed for adults, though with generally lower requirements. At $D = 0.25$, the optimum sample size ranged from 4 to 11 plants, increasing to 10 to 30 plants at $D = 0.15$ and 25 to 70 plants at $D = 0.10$ (Fig. 2, right column, up to down respectively).

For the combined population, sample size requirements were inversely related to pest density. At the lowest precision level ($D = 0.25$), as few as 4 plants were sufficient when densities exceeded 160 insects per shoot, whereas 12 plants were required at densities of approximately 1.6 insects per shoot. At higher precision levels, sample size demands increased considerably. For example, at $D = 0.15$, between 11 and 35 plants were required across a density range of 177 to 1.15 insects per shoot, while at $D = 0.10$, the optimum number of samples rose to 18 to 44 plants (Fig. 3, right column, up to down respectively).

Decision curves further indicated that, in most cases, reliable estimates of mean population density could be obtained with relatively few samples. On average, fewer than 5, 15, and 25 plants were sufficient to achieve precision levels of $D = 0.25$, 0.15, and 0.10, respectively, across developmental stages.

The relationship between mean density and optimum sample size was strongly negative for all life stages and precision levels (Fig. 3). At very low densities (<5 nymphs per shoot), the sample size required for adequate precision at $D = 0.25$ exceeded 1000 plants (Fig. 3, left column), reflecting the inherent challenge of accurately estimating sparse populations. However, as densities increased beyond 50 insects per shoot, required sample sizes declined sharply and stabilized at practical levels. Across all developmental stages, nymphs consistently required larger sample sizes than adults at comparable densities (Figs. 1 and 2, left column), reflecting the greater degree of aggregation observed in immature populations.

Sampling Plan Validation

Resampling validation confirmed the performance of FPSS plans for *P. solenopsis* at three precision levels ($D = 0.25$, 0.15, 0.10). Mean sample sizes for nymphs were 54, 150, and 338; for adults, 40, 111, and 250; and for combined stages, 47, 131, and 294, respectively (Fig. 4). Achieved precision closely matched target levels across all life stages. For combined stages, mean precision values were 0.269 (±0.04), 0.158 (±0.02), and 0.105 (±0.02), with success rates of 62%, 61%, and 59%, respectively (Fig. 5). Sample size was inversely related to population density. At low density (1.15 insects/plant), 312 (±45) samples were required for $D = 0.10$ (combined stages), decreasing to 198 (±8) at high density (176.85 insects/plant) (Table 2). The $D = 0.25$ plan significantly improved field efficiency, reducing mean sample size to 50 (±12) for combined stages (Table 3). Adults consistently required the fewest samples, followed by combined stages, then nymphs.

These validated plans are robust for density estimation. The $D = 0.10$ plan is suited for high-precision research, while $D = 0.25$ is optimal for management decisions.

Discussion

This study provides the first stage-specific sequential sampling plans for *P. solenopsis* on Chinese hibiscus in urban landscapes

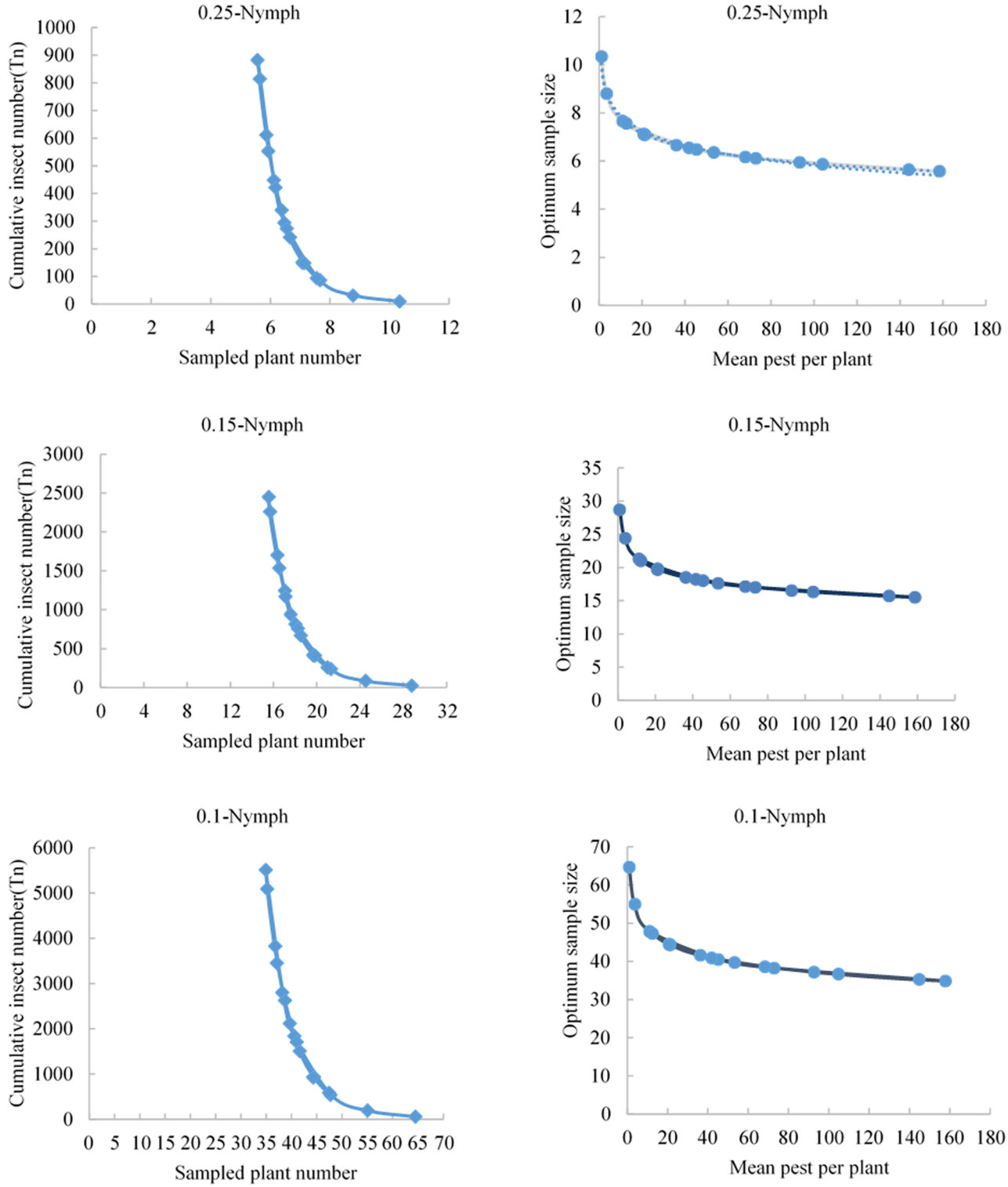

Fig. 1. The required optimum sample size (right) and fixed precision sequential sampling stop lines (left) based on Green's model of nymphs of *Phenacoccus solenopsis* on Chinese hibiscus at different precision levels (0.25, 0.15, and 0.1 from up to down, respectively).

of southwest Iran. Both TPL and IP regression indicated a strongly aggregated distribution, with higher aggregation in nymphs than in adults. This pattern reflects mealybug biology, where sedentary early instars remain clustered near maternal colonies, while adults disperse to new feeding sites (Beltrà et al. 2013, El-Zahi and Farag 2017, Boushi et al. 2020). The observed aggregation is consistent with previous findings for mealybugs on diverse hosts, including *Saccharicoccus sacchari* (Cockerell) on sugarcane (Allsopp, 1991), *Rastrococcus invadens* Williams in mango trees (Boavida et al. 1992), *Planococcus citri* (Risso) on citrus (Martínez-Ferrer et al. 2006), *Phenacoccus manihoti* Matile-Ferreroin in cassava fields

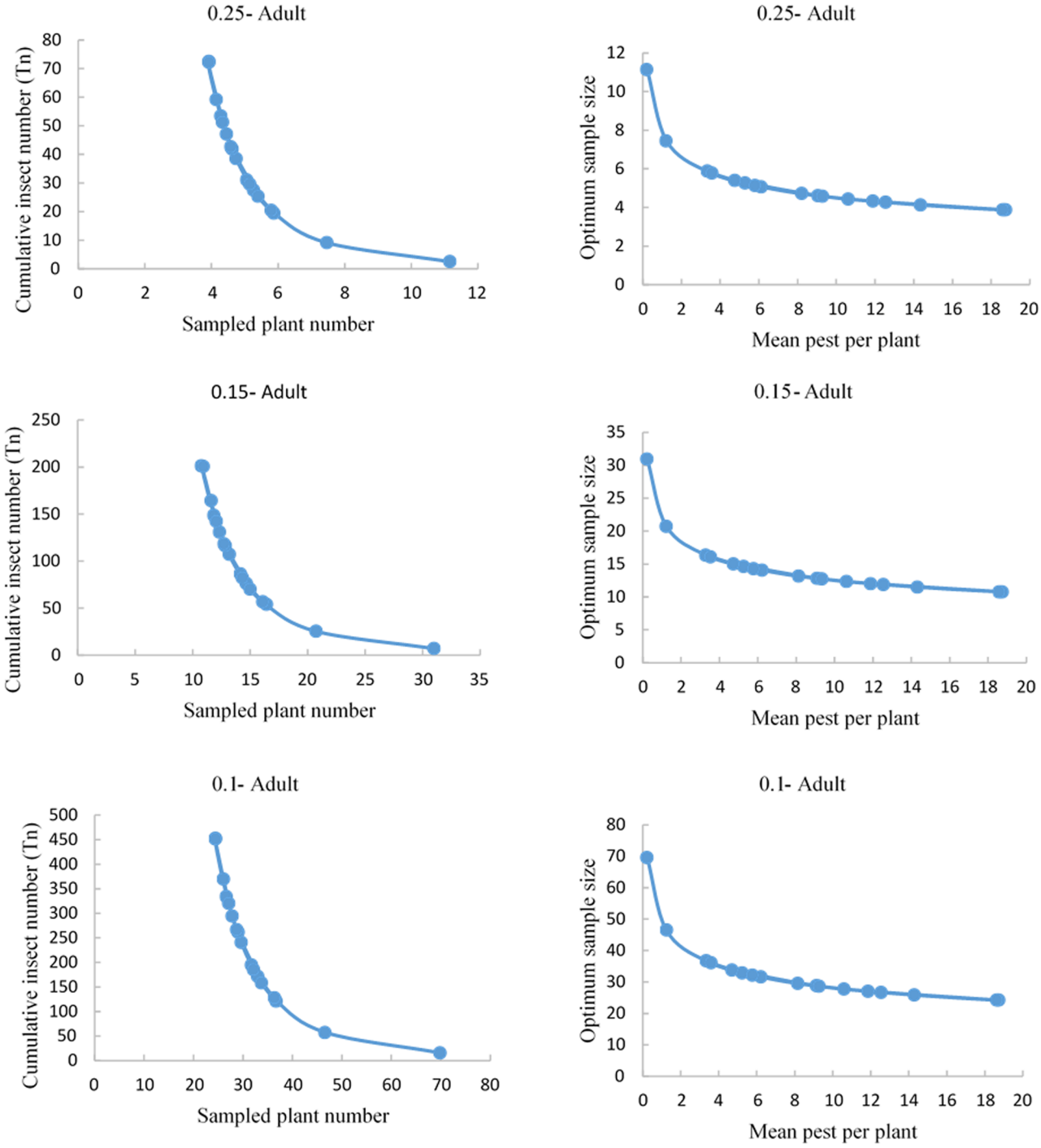

Fig. 2. The required optimum sample size (right) and fixed precision sequential sampling stop lines (left) based on Green's model of adults of *Phenacoccus solenopsis* on Chinese hibiscus at different precision levels (0.25, 0.15, and 0.1 from up to down, respectively).

(Schulthess et al. 1989), *P. peruvianus* Granara de Willink on bougainvillea (Beltrà et al. 2013), *Eriococcus ironsidei* Williams on macadamia (Gutierrez-Coarite et al. 2019), *Pseudococcus elisae* Borchsenius on banana (Becke et al. 2024) and *P. solenopsis* on cotton (Ahmad et al. 2011, Prasad et al. 2012). Comparable ontogenic shifts in spatial distribution have been observed in aphids (Hodgson et al. 2004, Fernandes et al. 2011, Severtson et al. 2016) and other sap-sucking insects, emphasizing the need to consider life stage when designing sampling plans.

The consistency of our TPL slope ($b = 1.82$ for combined stages) with values reported for cereal aphids (Ba-Angood and Stewart 1980, Ramezani et al. 2016), soybean aphid (Hodgson et al. 2004), cabbage aphid (Severtson et al. 2016) and the white mango scale insect, *Aulacaspis tubercularis* (Newstead) (Bakry and Abdel-Baky 2020) reinforces the universal tendency of hemipteran pests to exhibit aggregated distributions. The steep slopes of both models indicate a high degree of clumping typical of phloem-feeding, colony-forming insects with limited crawler dispersal (Taylor 1984, Wang et al. 2010). Such

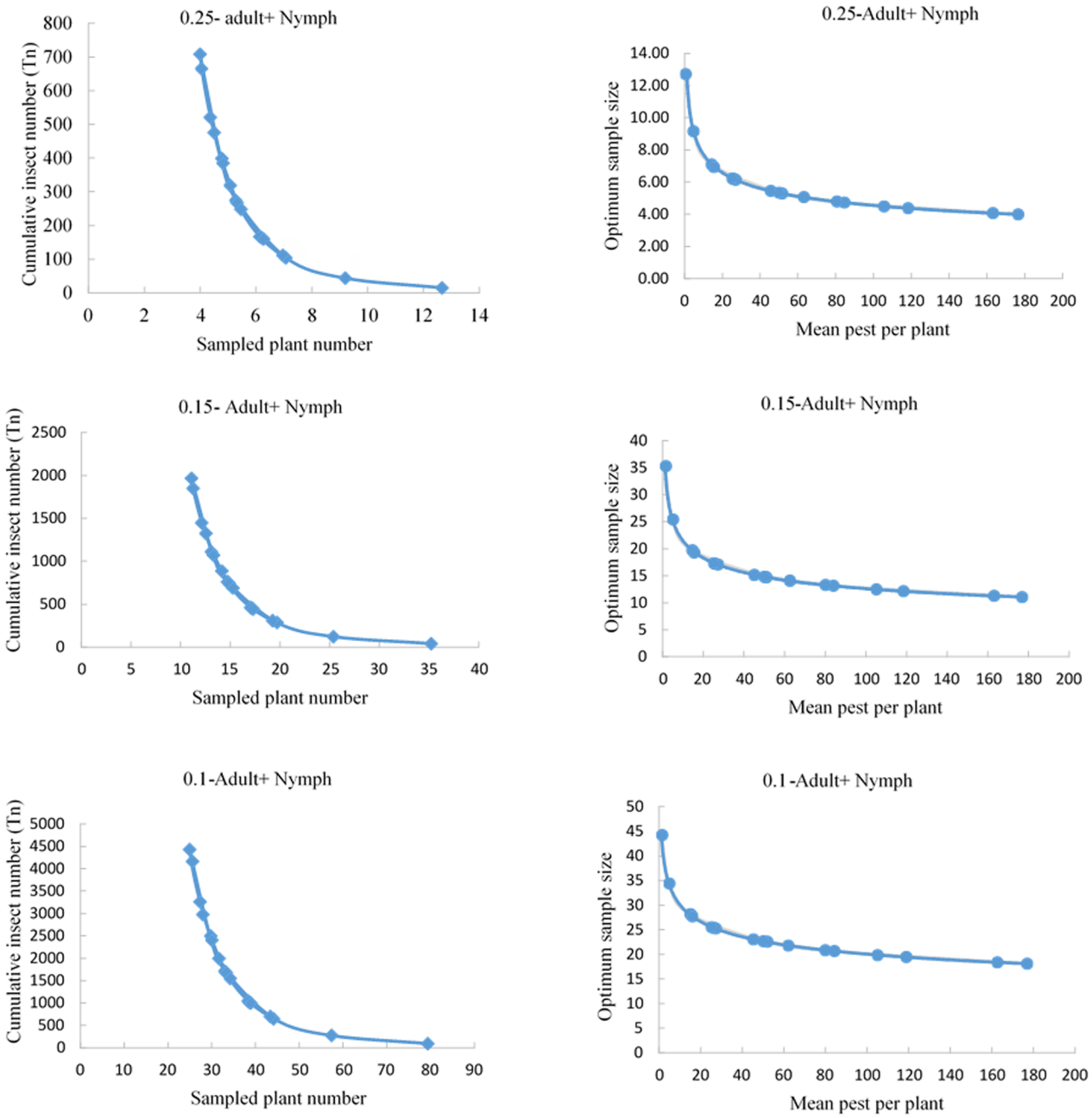

Fig. 3. The required optimum sample size (right) and fixed precision sequential sampling stop lines (left) based on Green's model of combined stage (adults + nymphs) of *Phenacoccus solenopsis* on Chinese hibiscus at different precision levels (0.25, 0.15, and 0.1 from up to down, respectively).

patterns also have ecological implications, as natural enemies exploit dense colonies more effectively, thereby enhancing biological control (Beltrà et al. 2013, Boushi et al. 2025).

The sequential sampling models developed here, based on Green's (1970) fixed-precision approach, markedly improved monitoring efficiency. Sample size requirements decreased with increasing population density, in accordance with theoretical expectations (Kapatos et al. 1996) and empirical findings for multiple arthropod pests (Ba-Angood and Stewart 1980, Hodgson et al. 2004, Fernandes et al. 2011, Severtson et al. 2016). At high densities, reliable estimates were obtained from fewer than five plants, whereas low densities required larger samples. This pattern mirrors results for *P. citri* and *P. peruvianus* (Martínez-Ferrer et al. 2006, Beltrà et al. 2013). Compared with pest systems that demand extensive sampling (Pérez-Rodríguez et al. 2017), our plans reduced effort by up to 75%, an important advantage in resource-limited urban settings. Nymphal populations required more samples than adults due to stronger aggregation, a trend also reported in other mealybug systems (Geiger and Daane 2001, Xu et al. 2024).

RVSP analysis confirmed that achieved precision levels closely approximated the targets ($D = 0.25$, 0.15, 0.10). These results are consistent with other sequential sampling validations (Pezzini et al. 2019, Mohseni Amin et al. 2024). Although approximately 60% of samples achieved exact target precision, the conservative bias minimizes the risk of underestimating pest densities, which

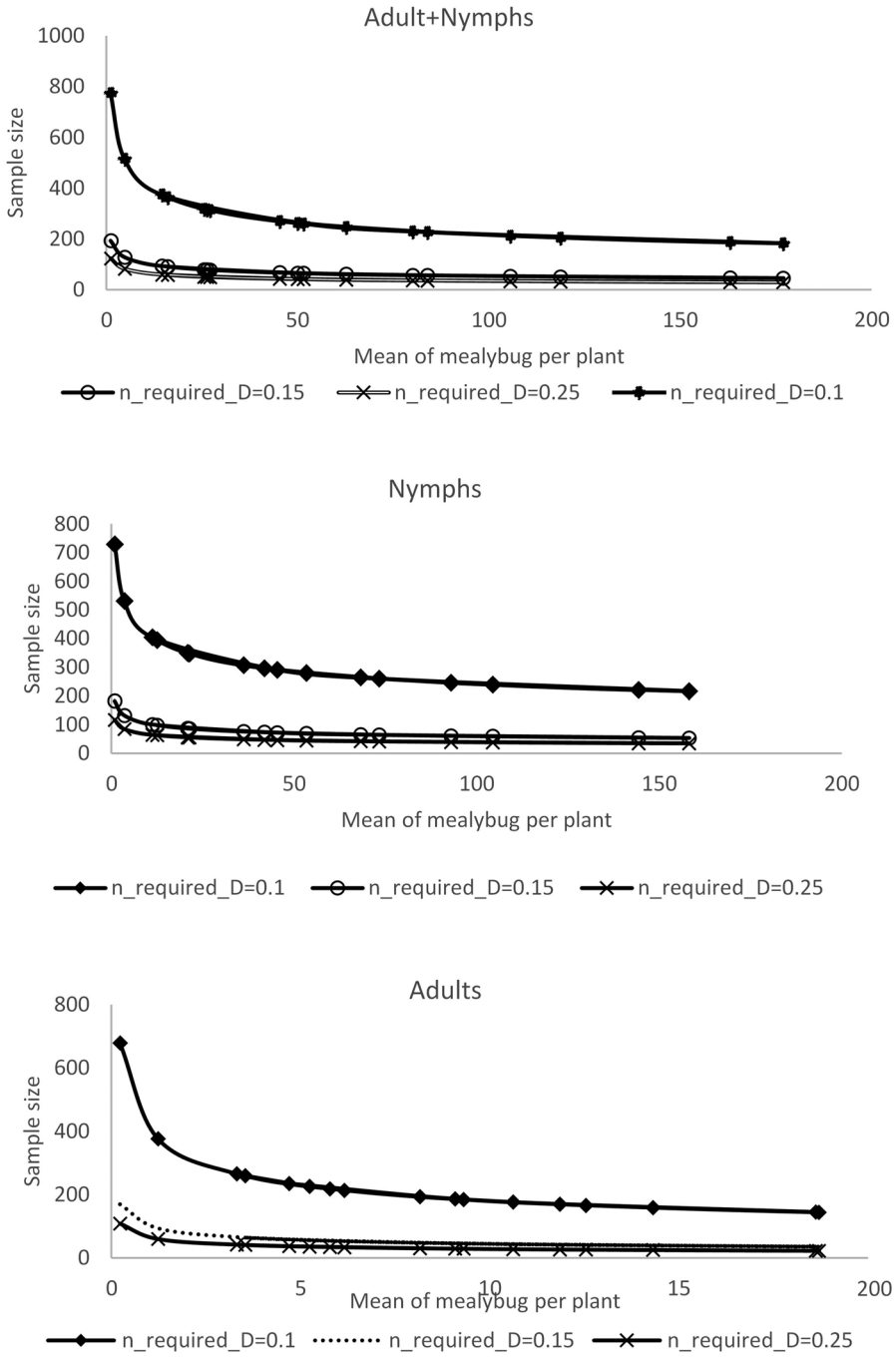

Fig. 4. Mean sample sizes (±SE) required by Green's sequential sampling plan to estimate *Phenacoccus solenopsis* density on Chinese hibiscus at three precision levels ($D = 0.25$, 0.15, and 0.10), derived from resampling validation of 10 independent data sets.

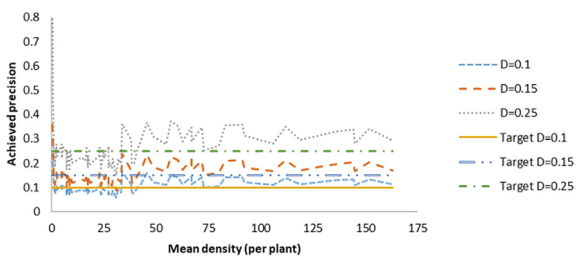

Fig. 5. Achieved precision (mean ± SE) from resampling validation of the sequential sampling plan for *Phenacoccus solenopsis* on Chinese hibiscus ($n = 10$ data sets) at three target precision levels ($D = 0.25$, 0.15, and 0.10).

is crucial for management reliability. The $D = 0.25$ plan provided strong accuracy with minimal field effort, ideal for operational IPM decisions, whereas $D = 0.10$ offered high precision for ecological or research applications—similar to efficient systems developed for *Aphis gossypii* Glover on cotton, *Brevicoryne brassicae* (L.) in canola field and cereal aphids in wheat field (Fernandes et al. 2011, Severtson et al. 2016, Ramezani et al. 2016). While our focus was on FPSS for detailed density estimation, fixed-sample-size or binomial classification plans may further streamline pest management. Such approaches have been successfully applied to *A. gossypii* and *Frankliniella schultzei* on cotton (Fernandes et al. 2011), and to stink bugs in soybean

Table 2. Resampling simulation used to validate the fixed-precision sequential sampling plan for *Phenacoccus solenopsis* (total number of adults + nymphs per plant) by using a preset precision level of 0.10 with replacement on hibiscus in Khuzestan province landscape

Average statistics for 100 sequential sampling simulations								
Data set	Observed density	Density mean	Precision (D)			Sample size		
			Mean	Max.	Min.	Mean	Max	Min.
1	1.15	1.18	0.11	0.12	0.10	312	378	200
2	4.77	4.81	0.10	0.11	0.09	267	321	200
3	14.54	14.62	0.12	0.14	0.11	398	482	200
4	16	16.25	0.13	0.15	0.12	425	508	200
5	26.23	26.60	0.11	0.13	0.10	319	385	200
6	50	50.35	0.10	0.11	0.09	263	315	200
7	51.54	51.23	0.09	0.10	0.08	231	265	200
8	83.92	84.51	0.13	0.15	0.12	445	531	200
9	105.54	106.21	0.08	0.09	0.07	225	254	200
10	176.75	175.92	0.07	0.08	0.06	198	223	200
Mean	53.05	53.61	0.10	0.12	0.09	308	366	200

Table 3. Resampling simulation used to validate the fixed-precision sequential sampling plan for *Phenacoccus solenopsis* (total number of adults + nymphs per plant) by using a preset precision level of 0.25 with replacement, on hibiscus in Khuzestan province landscape

Average statistics for 100 sequential sampling simulations						Sample size		
Data set	Observed density	Density mean	Precision (D)					
			Mean	Max.	Min.	Mean	Max.	Min.
1	1.15	1.19	0.26	0.29	0.24	50	61	32
2	4.77	4.83	0.25	0.27	0.23	43	52	32
3	14.54	14.67	0.28	0.32	0.25	64	78	32
4	16	16.25	0.31	0.35	0.28	68	81	32
5	26.23	26.60	0.27	0.31	0.24	51	62	32
6	50	50.41	0.25	0.28	.023	42	51	32
7	51.54	51.18	0.23	0.25	0.21	37	43	32
8	83.92	84.65	0.31	0.35	0.28	72	86	32
9	105.54	106.33	0.21	0.23	0.19	36	41	32
10	176.85	175.88	0.18	0.20	0.16	32	36	32
Mean	53.05	53.60	0.26	0.29	0.23	50	59	32

(Pezzini et al. 2019). Future research could adapt similar classification systems for *P. solenopsis*, or explore spatially optimized sequential sampling, as demonstrated for cabbage aphid in canola (Severtson et al. 2016), to reduce sampling effort by accounting for spatial autocorrelation.

Importantly, the conservative bias observed in our models minimizes the risk of underestimating pest densities, ensuring reliable decision-making within IPM programs. By tailoring sampling effort to pest density and life stage, these models provide efficient and cost-effective tools for managing *P. solenopsis*, while also supporting biological control and reducing reliance on chemical insecticides.

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Author Contributions

Leila Ramezani (Conceptualization [lead], Data curation [lead], Formal analysis [lead], Methodology [lead], Validation [lead], Writing—original draft [lead]) and Zohreh Khorsandi Kouhanestani (Formal analysis [supporting], Investigation [supporting], Methodology [supporting], Software [supporting])

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Conflicts of Interest

None declared.

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