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Research

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Sampling and Biostatistics

Spatial distribution and sequential sampling plan for *Oligonychus punicae* (Acari: Trombidiformes: Tetranychidae) on grapevine

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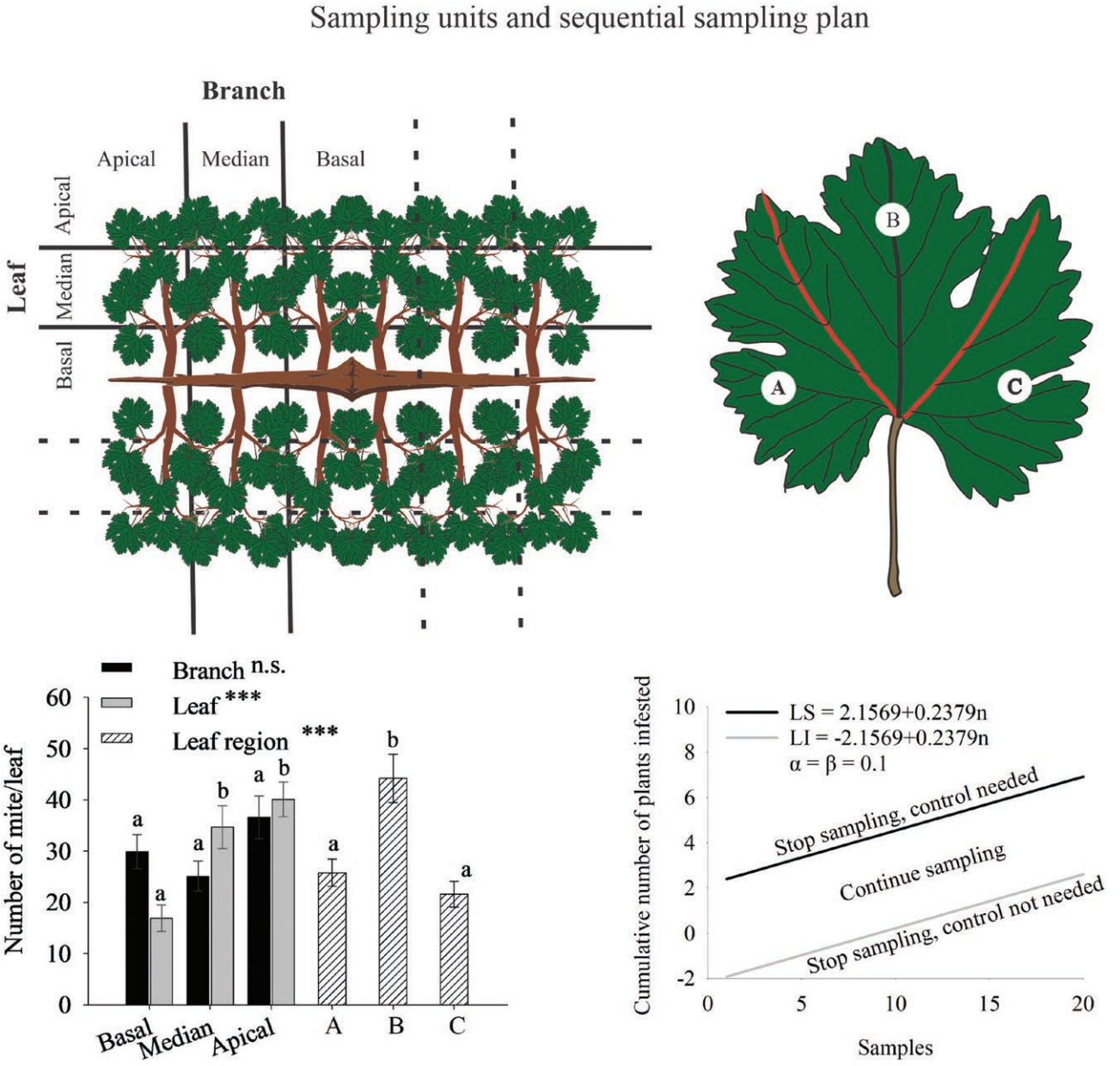
Sequential sampling plans are employed for the rapid characterization of infestations to facilitate decision-making. This study aimed to (i) investigate the spatial distribution of *Oligonychus punicae* (Hirst) in grapevine crops, (ii) determine the most representative branch, leaf, and leaf region for monitoring, and (iii) develop a sequential sampling plan for decision-making to control *O. punicae* in the table grape varieties Arra 15, BRS Vitória, Cotton Candy, Sugar Crisp, and Timpson at different phenological stages. *O. punicae* distribution was analyzed across all varieties and developmental stages. Notably, *O. punicae* distribution varied among the tested varieties, with no consistent pattern observed in branches and leaves. However, a distinct distribution pattern was evident within leaves, with larger populations concentrated in the central regions. The lower and upper economic thresholds implement control measures varied according to the phenological stage, with the lower thresholds (economic threshold) set at 36% and the upper thresholds (economic injury level) at 40%. Consequently, a minimum of 3 and maximum of 20 plants were sampled. Overall, this study provides a robust approach to optimizing resource allocation and minimizing the environmental impact of *O. punicae* management.

Key words: red spider mite, integrated pest management, sampling technique, action threshold

Graphical Abstract



Introduction

The red spider mite, *Oligonychus punicae* (Hirst) (Trombidiformes: Tetranychidae), is a cosmopolitan species found in 35 countries, including Africa, the United States, Asia, and Oceania (Migeon and Dorkeld 2024). This mite has been reported on 90 host plant species across 34 botanical families, with grapevines (*Vitis vinifera* L.) being among the most infested host (Domingos et al. 2014, Bragard et al. 2021, Mani 2022, Migeon and Dorkeld 2024). It causes significant economic damage and is typically controlled using pesticides (Bragard et al. 2021, Mani 2022). However, information on the spatial distribution and sampling of *O. punicae* in grapevine is currently lacking. This study, therefore, provides valuable data for sampling *O. punicae* and implementing a viable sampling plan within the integrated pest management (IPM) of *V. vinifera*.

Sampling is a fundamental component of IPM to assess the degree of infestation and estimate the threshold density of pests for decision-making in agriculture (Barzman et al. 2015, Onstad et al. 2019). Sampling in the field presents several challenges associated with sampling time, host phenology, sampling technique, and sampling unit (i.e., the number of plants and representative plant organs or structures) (Belien et al. 2019). Establishing an appropriate sampling plan, either conventional or sequential, is a critical aspect of decision-making (Bacci et al. 2008, Pedigo et al. 2021).

Sequential sampling is an important technique for efficiently estimate pest populations and is widely used in agriculture (Pedigo et al. 2021). Sequential sampling plans facilitate the precise determination of infestation, thereby reducing the time and effort required for sampling (Silva et al. 2014). Compared to conventional sampling, which relies on fixed samples, sequential sampling can be stopped once the required precision level is reached for real-time decision-making (Carleton et al. 2013). Decisions regarding whether to control pest populations are primarily based on calculations considering

the spatial distribution pattern of the pest (uniform, random, or aggregated), sampling unit, economic injury level (EIL), and economic threshold (ET) (Carleton et al. 2013, Benedetti et al. 2015).

Spatial distribution or degree of aggregation of a pest affects the sample size necessary to estimate or classify population densities and determine the appropriate monitoring strategy for specific areas, which can vary across varieties and phenological developmental stages of plants (Davis 2020). Therefore, this study aimed to (i) investigate the spatial distribution of *O. punicae* in grapevine crops, (ii) determine the most representative branch, leaf, and leaf region for monitoring, and (iii) develop a sequential sampling plan for decision-making to control *O. punicae* in the most cultivated table grape varieties in the study region, particularly those of greatest economic value, such as Arra 15, BRS Vitória, Cotton Candy, Sugar Crisp, and Timpson at different phenological stages, as these varieties have shown high infestations of *O. punicae* in the field.

Materials and Methods

This study was conducted on commercial table grape farms using the "T" trellis system in the São Francisco Valley, Pernambuco, Brazil, from December 2022 to September 2023. Sampling was carried out in plots of approximately 1.5 ha, each containing ~1.600 *V. vinifera* grapevine plants. For this study, plots consisting of the most cultivated grape varieties in the region, with 2 plots for each of the varieties Arra 15, BRS Vitória, Cotton Candy, Sugar Crisp, and Timpson, totaling 10 sampled plots. In addition, 3 plots were selected for each phenological developmental stage of the plant, which was measured in days after pruning (D.A.P.): vegetative stage (0–40 D.A.P.), flowering/fruiting stage (41–70 D.A.P.), and maturation/harvest stage (71–100 D.A.P.), totaling 9 sampled plots. Previously, specimens of *O. punicae* were mounted on microscope slides in medium Hoyer. Male and female mites were identified using specialized literature, such as manuals and identification key and a phase contrast microscopy, model Olympus BX41 (Bolland et al. 1998, Migeon and Dorkeld 2024, Moraes et al. 2024).

Spatial Distribution

The population occurrence data for *O. punicae* were fitted using frequency distribution models to Poisson (when the ratio between variance $[S^2]$ and mean $[\bar{X}]$of the number of mites equaled 1 $\left[\frac{S^2}{\bar{X}} = 1\right]$); negative binomial $[\frac{S^2}{\bar{X}} > 1]$; and positive binomial $[\frac{S^2}{\bar{X}} < 1]$. In addition, a chi-square degree of fit test was conducted to compare the observed distribution (data histogram) with the expected distribution (fitted distribution). Using a significance level of $P = 0.05$, we rejected the null hypothesis, indicating that the data do not follow a Poisson distribution but instead fit a negative binomial distribution (Luo and Liu 2024). *Oligonychus punicae* distribution was calculated using the *I* index (Rabinovich 1980), the aggregation index (*K*) (Elliott 1979), and Morisita's index ($I\delta$) (Morisita 1959). The *I* index measures the deviation from a random arrangement (ratio between variance and squared mean

$$\left(I = \frac{S^2}{\bar{X}^2}\right) \tag{1}$$

is commonly used to measure the deviation of an arrangement in random conditions, where $I = 1$ represents a random distribution, $I > 1$ represents an aggregated distribution, and $I < 1$ represents a regular distribution).

mThe aggregation index *K* is used only when the data indicate a negative binomial distribution:

$$K = \left[\frac{\bar{X}^2}{(S^2 - \bar{X})}\right] \tag{2}$$

As verified in this study, where a negative *K* represents regular dispersion; $0 < K < 2$ is highly aggregated distribution, $2 < K < 8$ is moderately aggregated, and $K > 8$ is random dispersion). The $I\delta$ index is used regardless of the sample unit size and is, therefore, a good index when the number of sample units is the same on the fields under comparison. It is determined as follows:

$$I_\delta = \frac{(\sum \bar{X}^2 - \sum \bar{X})}{(\sum \bar{X})^2 - \sum \bar{X}} \tag{3}$$

where $I\delta = 1$ indicates random dispersion, $I\delta > 1$ indicates aggregated dispersion, and $I\delta < 1$ indicates regular dispersion. In general, there was close agreement between these indices for use in this study.

Selection of Branch, Leaf, and Leaf Region

Branches, leaves, and regions of leaves to be sampled were selected based on the criteria of precision and representativeness to select the best sampling unit for representing the occurrence of *O. punicae* in different varieties (Rosado et al. 2014, Trumble 2020). The criterion of relative variance precision (*RV*) was used, with an $RV < 25\%$ indicating higher precision in the choice of the sampling unit (Bacci et al. 2008, Rosado et al. 2014). The relative density of *O. punicae* was defined as the number of mites sampled on the leaves, while the absolute density was defined as the total number of leaf mites sampled on the branches. Accordingly, sampling units were selected to ensure that the estimates obtained were both precise and representative of the mite populations on grapevine plants.

Ten grapevine plants were randomly sampled from each plot for observation. The plant canopy was divided into 3 distinct sections: basal, median, and apical branches (Fig. 1A). Leaves were identified by assigning sequential numbers, starting with the most apical leaf of the apical branch as number 1, followed by the median leaf as number 2, the basal leaf as number 3, and so on, on the median and basal branches. All leaves were divided into 3 regions, demarcated by the main veins dividing the leaves into 3 lobes: left "A," central "B," and right "C" lobes (Fig. 1B). Next, *O. punicae* density was evaluated using a pocket magnifier at 20× magnification by directly counting the adult mites and other mobile forms on the adaxial leaf surface. In total, 9 leaves per plant and 90 leaves per sample were evaluated, ensuring representative sampling of different parts and sections of grapevine plants.

Sequential Sampling Plan

EIL and ET

To determine the EIL, the cost of controlling *O. punicae* was estimated for the study region. This included the acaricide Abadin 72 EC (R$130.00 L^{-1}), applied with 3 sprays of 250 ml ha^{-1} per crop cycle, totaling R$90.00, as well as the equipment operational cost and operator labor, totaling R$30.00 ha^{-1}. The grape production value for the export market was estimated based on *O. punicae* density, using data from farm databases for the 2020, 2021, and 2022 harvests, with an average of R$33.11 kg^{-1}. The EIL was then determined using variables *N* (percentage of pests causing damage) and *I* (percentage of yield loss). The percentage of pests causing damage was based on the *O. punicae* infestation index relative to quality loss and product market value. It was estimated that a 20% infestation level of *O. punicae* leads to a 10–20% reduction in market value. EIL was determined as follows:

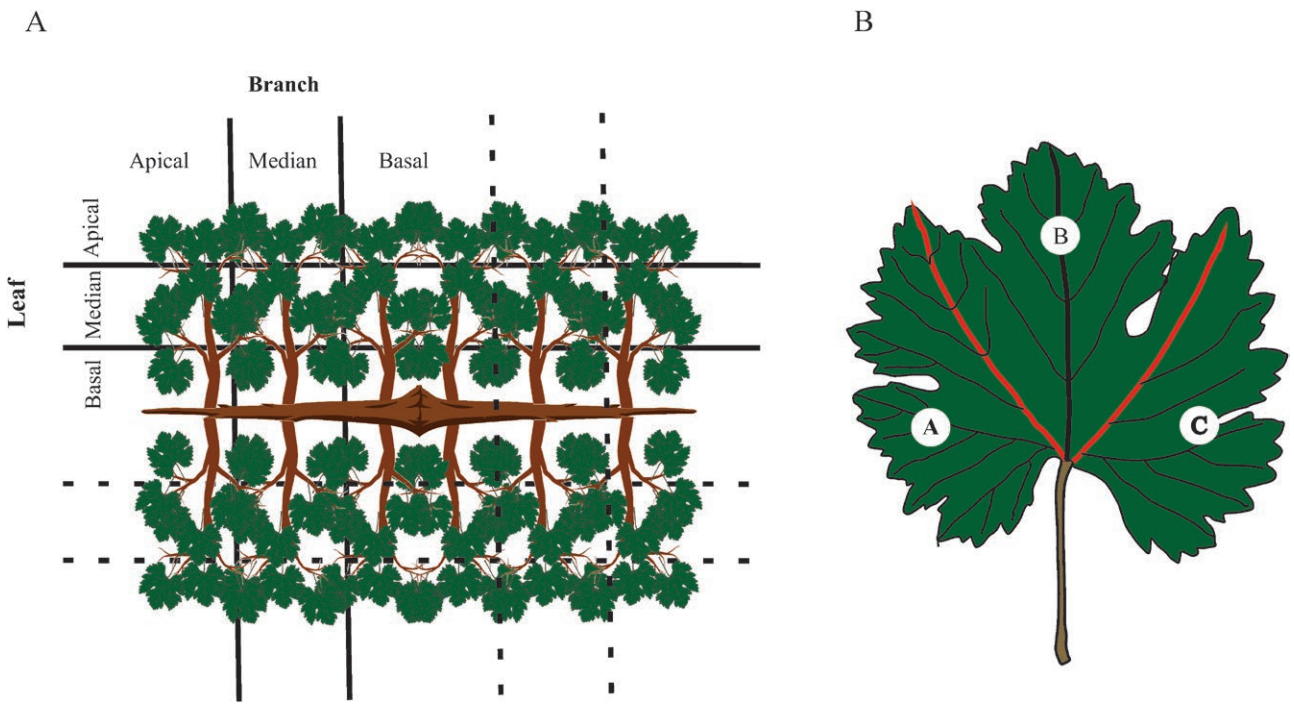


Fig. 1. Top view of the grapevine plant (T-trellis system) representing the sampling units (positions of the branch on the plant and leaf on the branch [A]) and leaf regions (bound by the main veins dividing the leaf into 3 lobes: left "A," central "B," and right "C" [B]).

$$\left(EIL = \frac{C \times N}{V \times I}\right) \quad (4)$$

where C = control cost per unit of production, N = percentage of the pest causing damage, V = market value per unit of production, and I = percentage of yield loss (Pedigo et al. 1996, Riley 2008). Control cost data included the value of the products (acaricides and adjuvants), equipment, and daily operator fees. ET was determined as follows:

$$ET = \frac{C \times D}{E}) \quad (5)$$

where C = control cost per unit of production, D = economic damage per unit of production, and E = expected control efficiency (80%) (Pedigo et al. 1996, Riley 2008).

Decision Thresholds (lower limit [LL] and upper limit [UL])

Probabilities of UL and LL were determined as follows:

$$UL = (ul = Y_1 + b \times n) \; and \; LL = (ll = Y_0 + b \times n) \quad (6)$$

where ul = maximum value of the UL, indicating a density equal to the control level, ll = maximum value of the LL, which does not cause economic damage, Y_0 and Y_1 = intercepts of decision lines, b = slope coefficient of the line, and n = number of samples collected or examined (Young and Young 1998). The slope coefficient was determined according to the dispersion pattern of *O. punicae* and fitted to a negative binomial model (aggregated dispersion) using the following equation:

$$b \times K \times \left(\frac{log\frac{q2}{q1}}{log\frac{p2\times}{p1}\frac{q1}{q2}}\right) \quad (7)$$

In this model, variables p_1 and p_2 were determined as follows:

$$p_1 = (m_1/K) \; and \; p_2 = (m_2/K) \quad (8)$$

where m_1 = α and β errors (considered in the model of 10) and m_2 = ET; K = aggregation index. Variables q_1 and q_2 were determined as follows:

$$q_1 = (1 - p_1) \; and \; q_2 = (1 - p_2) \quad (9)$$

The intercepts of decision lines were determined as follows:

$$Y_0 = \frac{log\left(\frac{\beta}{1-\alpha}\right)}{log\left(\frac{p_{2\times}}{p_{1\times}}\frac{q_1}{q_2}\right)} \text{ and } Y_1 = \frac{log\left(\frac{1-\beta}{\alpha}\right)}{log\left(\frac{p_{2\times}}{p_{1\times}}\frac{q_1}{q_2}\right)} \quad (10)$$

Data Analysis

Population density data of *O. punicae* used to select branches, leaves, and leaf regions to be sampled were analyzed using generalized linear models (GLMs), with the distribution adjusted to the Poisson model and overdispersion checked using the RT4Bio package and corrected using the quasi-Poisson model, as requires. Relative density was calculated as the number of adult mites and mobile forms per leaf in the branch and leaf regions (A, B, and C; Fig. 1B), whereas absolute density was indicated as the number of adult mites and mobile forms per branch. Phenological development stage, varieties, branches, and leaves were considered as explanatory variables in the model, while the relative and absolute number of mites served as response variables. The models were simplified when no significant interactions were found between variables using the chi-square test ($P \leq 0.05$). To verify differences in mite densities per branch, leaf on the branch, and leaf region, a contrast analysis was performed using GLM with statistical software R version 4.0.5 for Windows (R Core Team 2023). Data to estimate the ET and EIL indices, such as the percentage of *O. punicae* infestation, control costs, and market values of commercialized grapes, were obtained from farm databases. All models were analyzed using Microsoft Excel version 365.

Results

Spatial Distribution

Spatial distribution of *O. punicae* fitted the negative binomial model for all grapevine varieties and phenological stages ($P > 0.05$), showing high aggregation for the BRS Vitória, Cotton Candy, and Sugar Crisp ($0 < K < 2$) varieties and moderate aggregation for the Arra 15 and Timpson ($2 < K < 8$) varieties. Spatial distribution of *O. punicae* was highly aggregated ($0 < K < 2$) across all phenological stages. I_P index also indicated aggregated distribution for all varieties and phenological stages ($I_P > 1$; Tables 1 and 2).

Branch, Leaf, and Leaf Region Selection

Absolute mean density of *O. punicae* on branches differed only for Timpson ($\chi^2 = 1185$, $df = 186$, $P < 0.001$), with the highest mean number of mites found on the basal branch (29 ± 4.1 mites/branch). No significant differences were observed in the absolute densities of *O. punicae* on the branches of Arra 15 ($\chi^2 = 389$, $df = 537$, $P = 0.063$), BRS Vitória ($\chi^2 = 74.7$, $df = 1077$, $P = 0.547$), Cotton Candy ($\chi^2 = 297$, $df = 267$, $P = 0.050$), and Sugar Crisp ($\chi^2 = 133$, $df = 807$, $P = 0.230$; Fig. 2A–E). Absolute density of *O. punicae* on branches also did not differ significantly among the vegetative (0–40 D.A.P.; $\chi^2 = 133$, $df = 807$, $P = 0.230$), flowering/fruiting (41–70 D.A.P.; $\chi^2 = 96380$, $df = 277$, $P = 0.112$), and maturation/harvesting (71–100 D.A.P.; $\chi^2 = 195$, $df = 2886$, $P = 0.233$; Fig. 2F–H) stages.

Relative density of *O. punicae* on leaves differed among Arra 15 ($\chi^2 = 27396$, $df = 537$, $P < 0.001$), BRS Vitória ($\chi^2 = 76076$, $df = 1077$, $P < 0.001$), and Sugar Crisp ($\chi^2 = 12632$, $df = 807$, $P < 0.001$), but no significant differences were observed between Cotton Candy ($\chi^2 = 11460$, $df = 267$, $P = 0.860$) and Timpson ($\chi^2 = 79$, $df = 186$, $P = 0.406$). Mean mite counts were higher on the apical leaves of Arra 15 (40.0 ± 3.3 mites/leaf), median leaves of BRS Vitória (31.5 ± 2.4 mites/leaf), and basal leaves of Sugar Crisp (6.7 ± 1.1 mites/leaf). Relative density of *O. punicae* on leaves also differed significantly among the vegetative (0–40 D.A.P.; $\chi^2 = 12632$, $df = 807$, $P < 0.001$), flowering/fruiting (41–70 D.A.P.; $\chi^2 = 95896$, $df = 2076$, $P < 0.002$), and maturation/harvesting (71–100 D.A.P.; $\chi^2 = 127012$, $df = 2886$, $P < 0.001$; Fig. 2A, B, D, and F–H) stages. Mean mite counts were higher on basal leaves during the vegetative stage (0–40 D.A.P.; 6.7 ± 0.7 mites/leaf) and on median leaves during the flowering/fruiting (41–70 D.A.P.) and maturation/harvesting (71–100 D.A.P.) stages (30.2 ± 1.7 and 23.2 ± 1.3 mites/leaf, respectively).

Infestation density of *O. punicae* across leaf regions differed significantly among Arra 15 ($\chi^2 = 27680$, $df = 537$, $P < 0.001$), BRS Vitória ($\chi^2 = 46371$, $df = 1077$, $P < 0.001$), Cotton Candy ($\chi^2 = 10503$, $df = 267$, $P < 0.001$), and Sugar Crisp ($\chi^2 = 12840$, $df = 807$, $P < 0.001$) varieties, but no significant differences were observed in Timpson ($\chi^2 = 95$, $df = 186$, $P = 0.350$). In varieties with significant differences, average number of mites was higher in the central region of the leaf as follows: Arra 15 (40.2 ± 4.7 mites/lobe), BRS Vitória (34.2 ± 2.6 mites/lobe), Cotton Candy (37.6 ± 4.9 mites/lobe), and Sugar Crisp (6.7 ± 1.1 mites/lobe). This pattern remained consistent across different phenological stages: vegetative (0–40 D.A.P.; χ^2 =

Table 1. Pattern of dispersion and distribution of mite *Oligonychus punicae* in grapevine varieties at different phenological stages (D.A.P.) in the São Francisco Valley, Brazil. Variance (S^2), standard deviation (S), standard error (SE), relative variance (VR), dispersion index (index *I*), aggregation index (K), and Morisita's index (I_P) are shown.

Variety/ D.A.P.	Mites/leaf (±SE)	Mites/plant (±SE)	S^2	S	VR	*I*	K	I_P	Dispersion frequency	Distribution
Arra 15	92 ± 10.2	825 ± 121.8	296831.8	544.8	14.8	359.5	2.3	1.4	Bn[a]	Ag[b]
BRS Vitória	74 ± 6.1	663 ± 78.3	245341.1	495.3	11.8	370.0	1.8	1.5	Bn	Ag
Cotton Candy	71 ± 11.8	640 ± 165.4	300768.1	548.4	25.8	469.3	1.4	1.7	Bn	Ag
Sugar Crisp	13 ± 2.4	120 ± 26.0	20300.0	142.5	21.6	168.6	0.7	2.3	Bn	Ag
Timpson	59 ± 10.8	527 ± 92.5	59830.6	244.6	17.5	113.4	4.7	1.2	Bn	Ag
0–40	13 ± 1.0	120 ± 26.0	20300.0	142.5	21.6	168.6	0.7	2.3	Bn	Ag
41–70	60 ± 3.4	536 ± 48.8	252396.6	502.4	9.1	470.6	1.1	1.9	Bn	Ag
71–100	74 ± 4.4	669 ± 56.5	249130.7	499.1	8.2	361.4	1.9	1.5	Bn	Ag

[a]Bn, Negative binomial dispersion frequency.
[b]Ag, Aggregate distribution.

Table 2. Density (mean ± SE), degrees of freedom (df), and chi-squared test (χ^2) comparing observed and expected frequencies based on Poisson, negative binomial, and positive binomial distributions for *Oligonychus punicae* in grapevine varieties at different phenological stages (D.A.P.) in the São Francisco Valley, Brazil.

		Poisson		Negative binomial		Positive binomial	
Variety/D.A.P.	Density (mean ± SE)	df	χ^2	df	χ^2	df	χ^2
Arra 15	825 ± 121.8	19	$P < 0.001^{ns}$	19	$P = 0.536^{*}$	19	$P < 0.001^{ns}$
BRS Vitória	663 ± 78.3	39	$P < 0.001^{ns}$	39	$P = 0.949^{*}$	39	$P < 0.001^{ns}$
Cotton Candy	640 ± 165.4	10	$P < 0.001^{ns}$	10	$P = 0.096^{*}$	10	$P < 0.001^{ns}$
Sugar Crisp	120 ± 26.0	29	$P < 0.001^{ns}$	29	$P = 0.683^{*}$	29	$P < 0.001^{ns}$
Timpson	527 ± 92.5	6	$P < 0.001^{ns}$	6	$P = 0.987^{*}$	6	$P < 0.001^{ns}$
0-40	120 ± 26.0	29	$P < 0.001^{ns}$	29	$P = 0.683^{*}$	29	$P < 0.001^{ns}$
41-70	536 ± 48.8	106	$P < 0.001^{ns}$	106	$P = 0.699^{*}$	106	$P < 0.001^{ns}$
71-100	669 ± 56.5	77	$P < 0.001^{ns}$	77	$P = 0.656^{*}$	77	$P < 0.001^{ns}$

*Significant at $P < 0.05$.
[ns]Nonsignificant at $P > 0.05$.

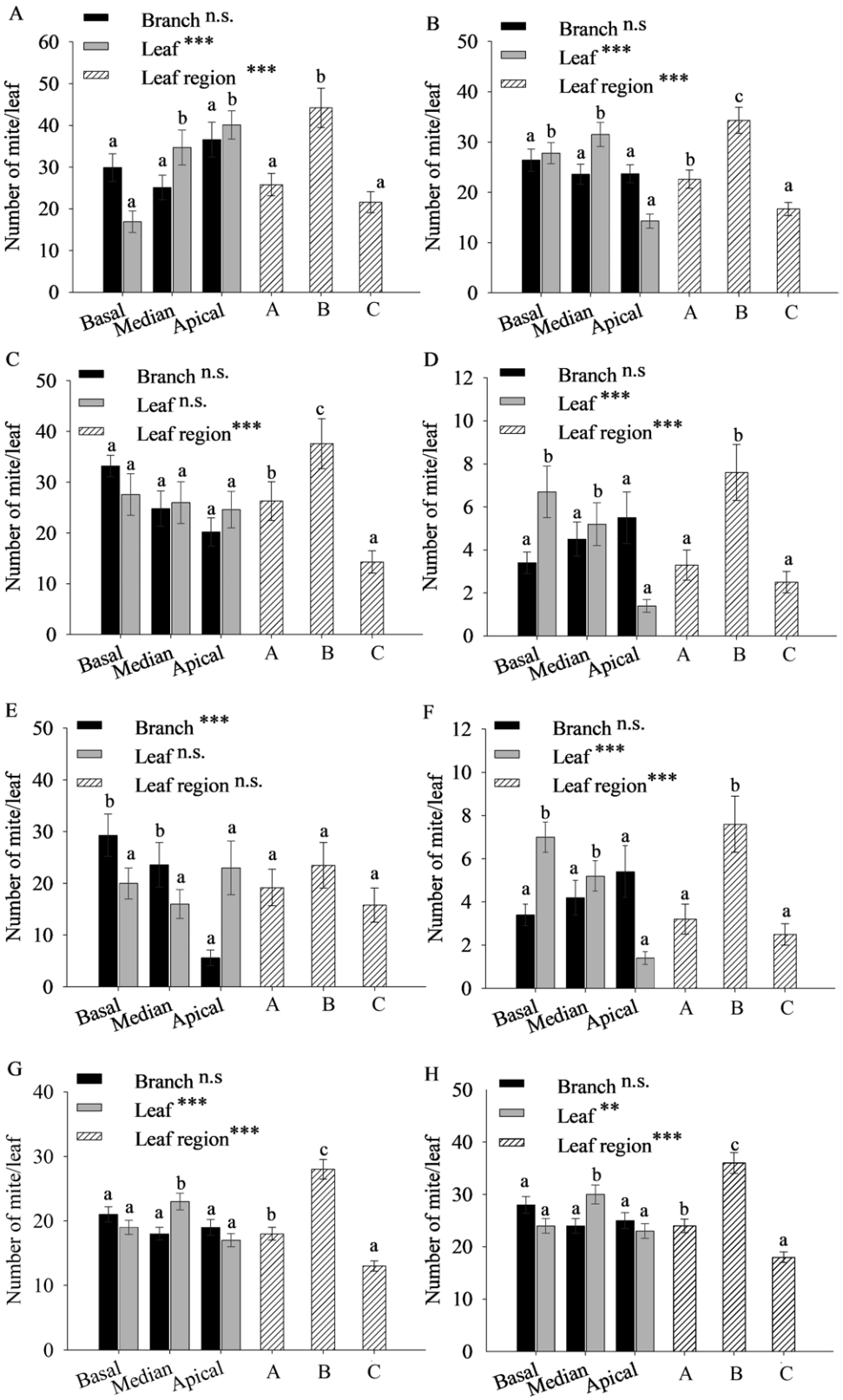


Fig. 2. Mean number of *Oligonychus punicae* on the branches (sum of mites on the leaves; black bars), leaf positions on the branches (gray bars), and lobes of the leaf regions (left "A," central "B," and right "C"; dashed bars) for the grape varieties (A) Arra 15, (B) BRS Vitória, (C) Cotton Candy, (D) Sugar Crisp, and (E) Timpson at different phenological stages (São Francisco Valley, Brazil). These stages are expressed in days after pruning (D.A.P.): (F) vegetative stage (0–40 D.A.P.), (G) flowering/fruiting stage (41–70 D.A.P.), and (H) ripening/harvest stage (71–100 D.A.P.). Asterisks indicate significant differences between the means for branches, leaves, and leaf regions. Different lowercase letters indicate significant differences between the means. "n.s" denotes no significant differences. Error bars represent the maximum and minimum confidence limits ($\alpha \leq 0.05$).

12840, df = 807, $P < 0.001$), flowering/fruiting (41–70 D.A.P.; χ^2 = 91842, df = 2076, $P < 0.001$), and maturation/harvesting (71–100 D.A.P.; χ^2 = 122412, df = 2886, $P < 0.001$). Higher populations of *O. punicae* were observed in the central region of the leaf (B), with 7.5 ± 1.3 mites/lobe during the vegetative stage (0-40 D.A.P.), 28.2 ± 1.5 mites/lobe during the flowering/fruiting stage (41–70 D.A.P.), and 36.6 ± 1.9 mites/lobe during the maturation/harvesting stage (71–100 D.A.P.; Fig. 2A–D and F–H).

Sequential Sampling Plan

EIL and ET

Economic thresholds for decision-making, defined as the pest infestation percentage causing yield loss, were estimated at 36% (ET) and 40% (EIL).

Decision Thresholds (LL and UL)

Intercepts of decision lines and slope coefficients ($-Y_0 + b$; $Y_1 + b$) were similar among varieties Arra 15 (Y_0 = –2.1 + 0.23; Y_1 = 2.1 + 0.23), Brs Vitória (Y_0 = –2.2 + 0.23; Y_1 = 2.2 + 0.23), Cotton Candy (Y_0 = –2.2 + 0.23; Y_1 = 2.2 + 0.23), Sugar Crisp: (Y_0 = –2.3 + 0.23; Y_1 = 2.3 + 0.23), and Timpson (Y_0 = –2.0 + 0.23; Y_1 = 2.0 + 0.23; Fig. 3A–E). Among the phenological development stages of the plant, Y_0 and $Y_1 + (b)$ values showed differences, with Y_0 = –2.8 + 0.22 and Y_1 = 2.8 + 0.22 for the vegetative stage (0–40 D.A.P.), Y_0 = –2.1 + 0.23 and Y_1 = 2.1 + 0.23 for flowering/fruiting stage (41–70 D.A.P.), and Y_0 = –2.2 + 0.23 and Y_1 = 2.2 + 0.23 for maturation/harvesting stage (71–100 D.A.P.; Fig. 3F–H). Reference values for LLs and ULs for all varieties ranged from 0 to 3 and 2 to 7, respectively. For the phenological development stages, LLs and ULs varied from 0 to 3 and 4 to 7 for the vegetative stage (0–40 D.A.P.), 0 to 3 and 3 to 7 for flowering/fruiting stage (41–70 D.A.P.), and 0 to 3 and 2 to 7 for maturation/harvesting stage (71–100 D.A.P.), respectively.

Discussion

The spatial distribution of a pest organism can influence requirements for sample size, sampling units, and walking direction when estimating and classifying population densities in the field (Davis 2020). *Z*-shaped walking patterns are recommended for organisms with random distribution, continuous walking for those with uniform distribution, and *U*-shaped walking for those with aggregated distribution (Wangchuk et al. 2022). In this study, spatial distribution of *O. punicae* in grapevines was in an aggregated, regardless of the variety or developmental stage of the crop. Other studies have shown that the spider mite *O. punicae* has an aggregated distribution on avocado (*Persea americana* Mill.; Lauraceae) (Lara-Vázquez et al. 2023) and eucalyptus (*Eucalyptus urophylla* S.T. Blake) plants (Ferraz et al. 2020). This aggregated distribution pattern is likely an ecological characteristic of the Tetranychidae family (Walter et al. 2013). Additional sampling studies, including U-shaped, Z-shaped, stratified, or systematic methods, should be conducted to determine whether the aggregation pattern of *O. punicae* influences the recommended sampling route.

Branches can be selected from any section of the canopy as the absolute density of *O. punicae* does not differ significantly among different sections. In vineyard agroecosystems, plant canopies are continuous, promoting the horizontal dispersal of mites within the vineyard (Aguilar-Fenollosa et al. 2016). The selection strategy for the most representative leaf on a branch may vary among different plant varieties and phenological stages of development. Basal leaves of the branch are more representative in the vegetative stage (0–40 D.A.P.), whereas median leaves are more representative in the reproductive stage (41–100 D.A.P.) of the plants. In addition, mite counts on the leaves should focus on the area around the central vein, where the highest population density was observed (Fig. 1b). Ferraz et al. (2020) also found that *O. punicae* populations are higher along the central vein of basal eucalyptus leaves. The prominent veins between the central vein and leaf blade may favor infestation by tetranychid mites by providing favorable conditions for web construction, shelter, and oviposition (Mushtaq et al. 2023).

Decisions regarding the use of acaricides in commercial grapevine cultivation in Brazil are often based on very low infestation levels (10%) (Botton et al. 2015). This promotes the excessive use of chemical pesticides, leading to the death of natural enemies, such as Phytoseiidae mites, thereby favoring the selection and spread of pesticide-resistant mite populations in the field (Ferreira et al. 2015, Monteiro et al. 2015, Glinushkin et al. 2019, Silva et al. 2019). Pesticide-resistant pest arthropod populations tend to expand and spread into previously unoccupied areas, thereby dispersing resistance genes and making control more challenging (Hawkins et al. 2019). This study, sequential sampling plans were developed, with a calculated ET of 36%. This threshold is consistent with that reported by Baillod et al. (1993), who used an ET of 20–40% for the control of the 2-spotted spider mite (*Tetranychus urticae* Koch) in grapevine plants.

In sequential sampling plans, the number of samples (plants) decreases as mite infestation intensity increases; however, in cases of low infestation, management decision-making is not prohibitive. Lower α and β rates reduce the expected errors of sampling plans; however, these must be weighed against the cost of increasing sample size for control decision-making (Alyousuf et al. 2021). In this study, *O. punicae* infestation intensity increased with the phenological developmental stage of the plant, influencing the mite monitoring dynamics over time. Previous studies have shown that *O. punicae* has a greater preference for older leaves, with higher population levels observed during the reproductive stage of mango plants (Melo et al. 2023, Melo et al 2024). Therefore, specific sampling plans are necessary for each phenological development stage of grapevine (D.A.P.), with a minimum of 3 to a maximum of 20 plants per sampling. In addition, a new sampling should be conducted after 8 days, given that the development cycle of *O. punicae* can be completed within 8 to 11 days on grapevine plants (Vásquez et al. 2008). Thus, this sampling proposal has a significant impact on the results obtained, particularly in population dynamics studies and management decision-making. However, we emphasize the importance of developing sampling plans that take plant phenological stages into account (such as young or old leaves), as this approach is more effective for predicting and controlling infestations.

In conclusion, this study provides monitoring information to accurately determine *O. punicae* population densities and implement a feasible sampling plan for IPM of *V. vinifera*. Our results indicate that mites should be directly counted in the fields on 9 leaves from specific regions of the plant, with 3–20 plants per sampling, rather than conducting fixed sampling of many randomly chosen leaves. Our study provides a robust approach for *O. punicae* management, enabling more efficient allocation of resources, minimizing the environmental and economic impacts, and increasing the effectiveness of control measures. However, further studies are necessary to assess the distribution of *O. punicae* and select sampling units with other planting systems, such as vertical trellises. In addition, more studies are needed to validate the sequential sampling methodology used for *O. punicae* in this study. For this, other statistical models could be used, such as calculating operating characteristic curves, mean number of samples (ASN) and sequential probability ratio test.

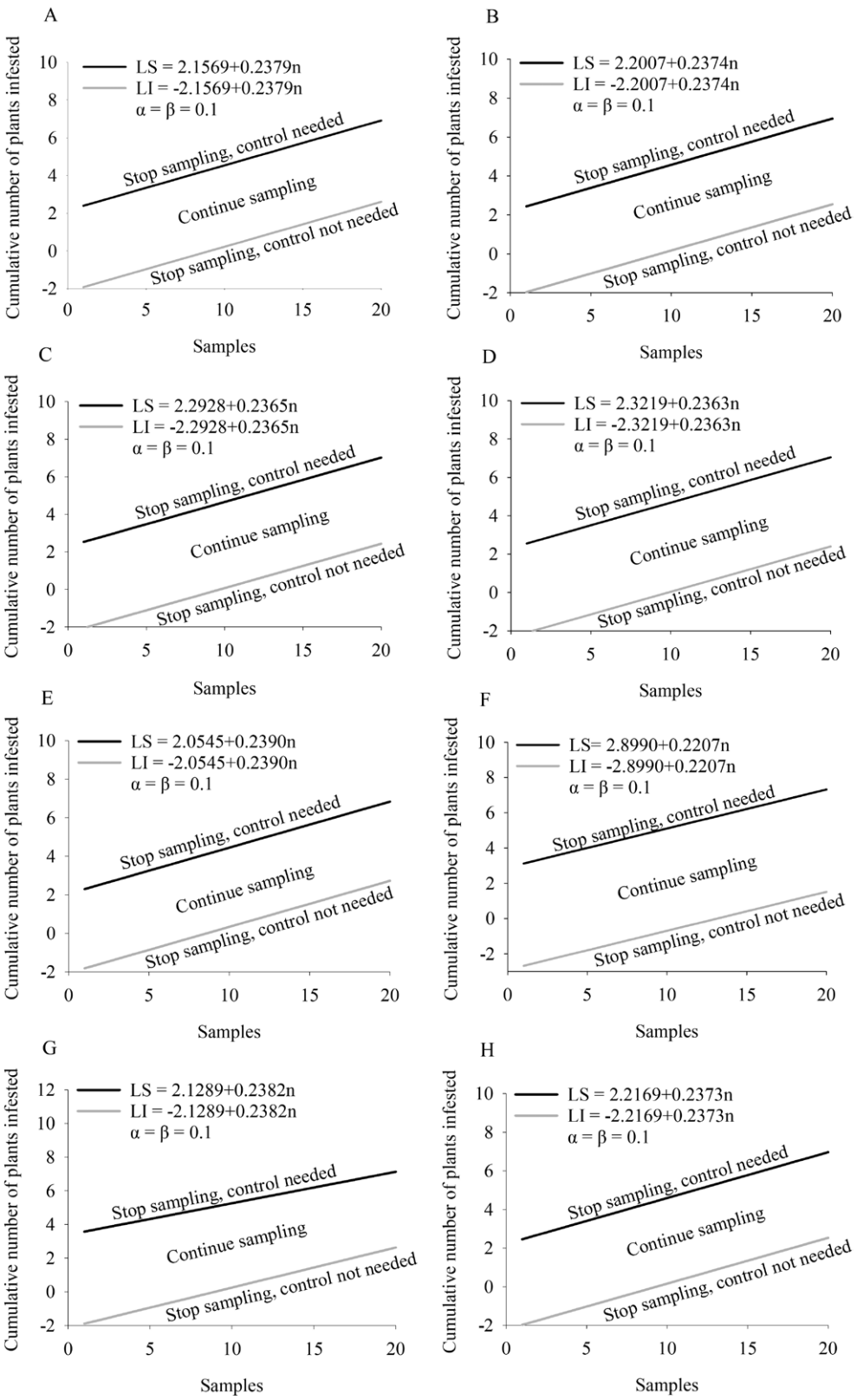


Fig. 3. Decision stop lines for the negative binomial sequential sampling plan for *Oligonychus punicae* for the grape varieties (A) Arra 15, (B) BRS Vitória, (C) Cotton Candy, (D) Sugar Crisp, and (E) Timpson at different phenological stages (São Francisco Valley, Brazil). These stages were measured in days after pruning (D.A.P.): (F) vegetative stage (0-40 D.A.P.), (G) flowering/fruiting stage (41–70 D.A.P.), and (H) ripening/harvest stage (71–100 D.A.P.). The plan is based on an action threshold of 36% of plants infested with the mite. Individual sampling units consisted of 20 consecutive grapevine plants. Sequential stop lines were calculated with error rates α and β set at 10% ($\alpha = \beta = 0.1$).

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