

Within-Plant Distribution and Binomial Sequential Sampling Plan for *Tetranychus evansi* (Acari: Tetranychidae) in Greenhouse Tomato: Implications for Management

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Abstract: The red spider mite, *Tetranychus evansi* is a critical pest of tomato in the tropics. Control of *T. evansi* has traditionally depended on acaricide treatments. However, it is only in a handful of crops where monitoring techniques for mites, using statistical methods, have been developed to help farmers decide when to spray. The objective of this study, therefore, was to develop a sampling plan that would help farmers increase accuracy, and reduce the labor and time needed to monitor *T. evansi* on tomato. The distribution of *T. evansi* within-plant was aggregated, and intermediate leaves (YFL) was the most appropriate sampling unit to evaluate the mite density. Analysis based on Taylor's Power Law showed an aggregated pattern of distribution of *T. evansi*, while assessment of the fitness of the binomial model indicated that a tally threshold of 5 mites per YFL provided the best fit. Consequently, binomial sequential sampling plans premised on three action thresholds (0.1, 0.2 and 0.3) were developed. The binomial sequential sampling plan for *T. evansi* developed in this study has the potential to significantly increase the chance for targeted acaricide applications. This judicious use of pesticides is especially crucial within the context of integrated pest management (IPM).

Keywords: arthropod pest; *Solanum lycopersicum*; red spider mite; pest management

1. Introduction

Arthropod pests severely constrain tomato production in the tropics. Among the major arthropod pests of tomato, *Solanum lycopersicum*, is the red spider mite, *Tetranychus evansi* Baker and Pritchard [1-3]. Spider mites are highly polyphagous and have been reported on various host plant species [4, 5]. Their high reproductive potential coupled with their rapid development results in a fast population increase [6, 7].

Control of spider mites has traditionally depended on acaricide treatments [2]. However, it is only in a handful of crops where monitoring techniques for mites, using statistical methods, have been developed to help farmers decide when to spray [8]. According to Dreves (1996), aided by an effective monitoring program, a farmer should be able to decide whether pests are present in sufficient numbers to trigger specific pest control action (e.g. pesticide application). Moreover, such pest monitoring programs should be readily accessible to farmers and adapted to their circumstances [9]. The objective of this study, therefore, was to develop a sampling plan that would help farmers increase accuracy, and reduce the labor and time needed to monitor *T. evansi* on tomato. Developing such a plan will increase the chance for targeted acaricide applications, ensuring application only when and where needed. This judicious use of pesticides, advocated by The International Code of Conduct on the Distribution and Use of Pesticides [10], is especially crucial within the context of integrated pest management (IPM).

In developing a monitoring program for arthropod pests, there is need to identify a sampling unit. Furthermore, the procedure for sampling and a sampling design should all be defined, and the efficiency and accuracy, all of which are often competing needs, should also be addressed [11]. Finally, an “action threshold” should be applied to inform a decision on whether an intervention is necessary or not [12].

2. Materials and Methods

2.1. Study area

The study was carried out in high tunnels at Dudutech’s Ladybird Farm during two growing seasons of 2015. The farm is located along the shores of Lake Naivasha, Nakuru County, Kenya (1,890 m above sea level). The high tunnels (50 m long, 10 m wide, and 4.5 m high) used for the study were enclosed by a single layer of 150-micron clear polythene that ensured the provision of adequate natural light. Furthermore, the tunnels were kept free of pesticide contamination, and for ventilation, they had sides that could be raised up to a height of 1 m. The soil in the high tunnels was sandy to loam soil (topsoil was mostly sandy with poor water retention capacity) and water to the tunnels was applied through drip-irrigation. During the duration of the study, the weather station at the farm recorded an average monthly maximum temperature of 27 °C, an average monthly minimum temperature of 11 °C and 12h photoperiod. The high tunnels were adjacent to each other, hence subjected to same climatic conditions.

The strain of *T. evansi* used in the study originated from a natural infestation of tomato plants at a farm within Naivasha, Kenya. The collected mites were reared on tomato plants in a rearing unit at Dudutech. The rearing unit for red spider mite was a 50 x 10 x 4.5-m high tunnel enclosed by a single layer of 150-micron clear polythene.

2.2. Within-plant distribution of *T. evansi*

Experiment was carried out in a single high tunnel. The growing spaces in the tunnel was divided into four 20 x 1 m raised beds (manually made along its axis). Seedlings of Rio-Grande tomato variety, procured from Longonot Farm propagation nursery, were sown per hole at a depth of 0.5 cm and thinned 2-3 days to maintain a tomato plant population within each bed at 192 plants. After that, tree poles were erected about 5cm away from each tomato hill for support. The plants were then inoculated (2 weeks after transplanting) with a population of 30 mobile *T. evansi* per plant.

Preplant of broadcast double super phosphate (DSP) fertilizer was applied one teaspoonful per point. When plants attained a height of 25cm, they were top dressed, applying 16 kg of calcium ammonium nitrate (CAN) per 0.4 acres then 64 kg 4 weeks later. Plots were essentially kept weed-free by hand-weeding.

The vertical distribution of *T. evansi* on tomato leaves was determined using a destructive sampling technique. Also, for purposes of this study, the host plants were maintained in a one-stem system. Data was collected on 4 different time periods (7, 21, 35- and 49-days following inoculation of the plants with *T. evansi*). During data collection, on the 4 different time periods, 24 plants were randomly selected from each of the 4 beds (96 plants in total). From each of the selected plants, leaves were then segregated into: “young leaves” (YL) (uncurled); “intermediate leaves” (YFL) (partly uncurled); and “mature leaves” (ML) (fully expanded). The average mite count per YLs, YFLs, and MLs, per plant was subsequently established.

The population of mobile *T. evansi* per sampling unit was established by counting the mites under a stereomicroscope [13]. For heavy infestations, mite counts were made following the procedure prescribed by Premachandra et al. (2005). Following the procedure, the tomato leaves were washed 3 times for ≈ 10s inside a plastic box (15 x 9 cm) filled with 250ml of 70% ethanol. Afterwards, the solution containing *T. evansi* was poured into a conical flask (200ml), shaken, and kept for a few minutes to allow the mites to settle. Once the mites inside the conical flask settled, the supernatant was decanted gently to 50ml. The suspension remaining inside the conical flask was then discharged onto a graduated plate and mite counts (larvae, nymphs and adults) made under a

stereomicroscope. Resulting from both counting mechanisms, the mite counts were presented as mean (m) $\pm$ standard error of the mean (SEM). Levene's test of equality of variance [14] was used to test the assumption of homogeneity of variance, and where there was a violation of the same, a modified version of ANOVA, Welch ANOVA, was used to analyze differences among the group means while Games-Howell post hoc test [15] was used to compare all possible combinations of group means.

2.3. Binomial sequential sampling plan

A binomial sequential sampling plan was selected since, unlike a fixed-sample-size plan, it requires fewer samples. In addition, binomial plans do not require the person sampling to count all the mites in each sampling unit. Instead, one is only required to establish whether the number of mites in a sample exceeds a defined threshold [16]. Assessment of sequential sampling plans are best carried out using the average sample number and the operating characteristic (i.e. the proportion of sampling instances over a series of pest densities resulting in a no intervention outcome). The operating characteristic is a gauge of the error of the decisions while the average sample number (i.e. the average number of sampling units obtained over a range of pest mean densities) is a gauge of sampling effort [8].

The experiment to develop a binomial sequential sampling plan for *T. evansi* on tomato was a complete randomized factorial arrangement of 2 inoculation rates ('mild infestation' and 'light infestation' inoculation rates), and 3 tomato varieties ('Ann F1', 'Tylka F1', and 'Rio-Grande') with 4 replications. The three commercial tomato varieties were chosen on account of being among the most representative of varieties cultivated in the country [17].

Two high tunnels were used for this experiment, a tunnel each for the different inoculation rates ('mild' and 'light'). The growing spaces in the respective tunnels were divided into four 40 x 2m raised beds (manually made along its long axis). Each bed was further divided into twelve 2m² (2 x 1 m) plots. Each of the 4 replications consisted of 4 plots per tomato variety, giving a total of 12 plots per replication. There was a single row per plot with 1m between plots and 50cm between plants. Seedlings, procured from Longonot Farm propagation nursery, were sown per hole at a depth of 0.5cm and thinned 2-3 days to maintain a tomato plant population within each plot at 6 plants (which totaled 24 plants per tomato variety per replication). After that, tree poles were erected about 5cm away from each tomato hill for support.

Two weeks after planting, the plants in both tunnels were inoculated with *T. evansi*. In the first tunnel, the plants were inoculated with a population comprising 130 mobile *T. evansi* (excluding eggs) (mild inoculation rate) while in the second tunnel, the plants were inoculated with 30 mobile mites (light inoculation rate).

Following inoculation of the mites, intermediate leaves (YFL) (the canopy stratum identified in the previous section as being ideal for assessing *T. evansi* population) from 16 plants (4 plants per plot) from each tomato variety, and from each of the tunnels, were destructively sampled on 4 different time periods (7, 21, 35- and 49-days following inoculation with *T. evansi*). The average count of mobile mites found on the intermediate leaves (sampling units examined, n) of each plant were made following the procedure previously described [18] and presented as mean $\pm$ standard error of the mean (SEM).

The association between the proportion of samples infested with *T. evansi* and the mean was determined using the procedure prescribed by Wilson et al. (1983):

$$PI = 1 - e^{-(m \ln(am^{(b-1)})/(am^{(b-1)}-1))} \quad (a)$$

in which PI is the proportion of sampling units infested with *T. evansi*, m is the average number of *T. evansi* per sampling unit, and a and b are Taylor's power law coefficients. Taylor's power law [19] describes the association between the variance (S^2) and the mean (m):

$$S^2 = am^b$$

where a = the intercept (sampling-dependent factor), and b = the slope (an aggregation index). Equation a can be simplified and expressed as follows [16]:

$$\ln(1 - PI) = -m \ln(am^{b-1}/(am^{b-1} - 1)) \quad (b)$$

The fitness of the binomial model was assessed through regression analysis where the left and right-hand sides of equation b were matched against each other. Curvilinear regression was performed using SPSS Statistics procedure.

The binomial model was extended to consider the proportion of sampling units with more than x mites per sampling unit, where $x = 5, 10$ and 20 . The level of x is known as the tally threshold (TT). Increasing TT increases the robustness of the sampling plan thereby making the plan less sensitive to variation [8].

The intercepts and slopes of the sequential plans were determined for the binomial distribution [20]. Like the tally thresholds, three action thresholds were selected (10, 20 and 30% of sampling units infested with at least 5 *T. evansi*) based on sensory thresholds, recommended by experienced crop scouts. For all the action thresholds, the nominal α and β error values were set at 0.05. The decision boundaries (lower and upper) were calculated [20] with the upper line values being rounded up while the lower line values being rounded down.

2.4. Estimation of population density

Estimation of *T. evansi* populations on a plant area base was determined using a generalized formula of the model presented in equation c [21].

$$\text{Mite density}/m^2 = m_s \beta^{-1} \gamma^{-1} \rho \lambda^{-1} \quad (c)$$

where:

m_s = projected mite density per sampled sampling unit,

β = number of mites on the sampled sampling units as a fraction of the entire number on sampling units,

γ = number of mites on the sampling unit as a percentage of the entire number on all leaves,

ρ = number of plants per meter-row, and

λ = spacing between rows (i.e. number of rows per meter).

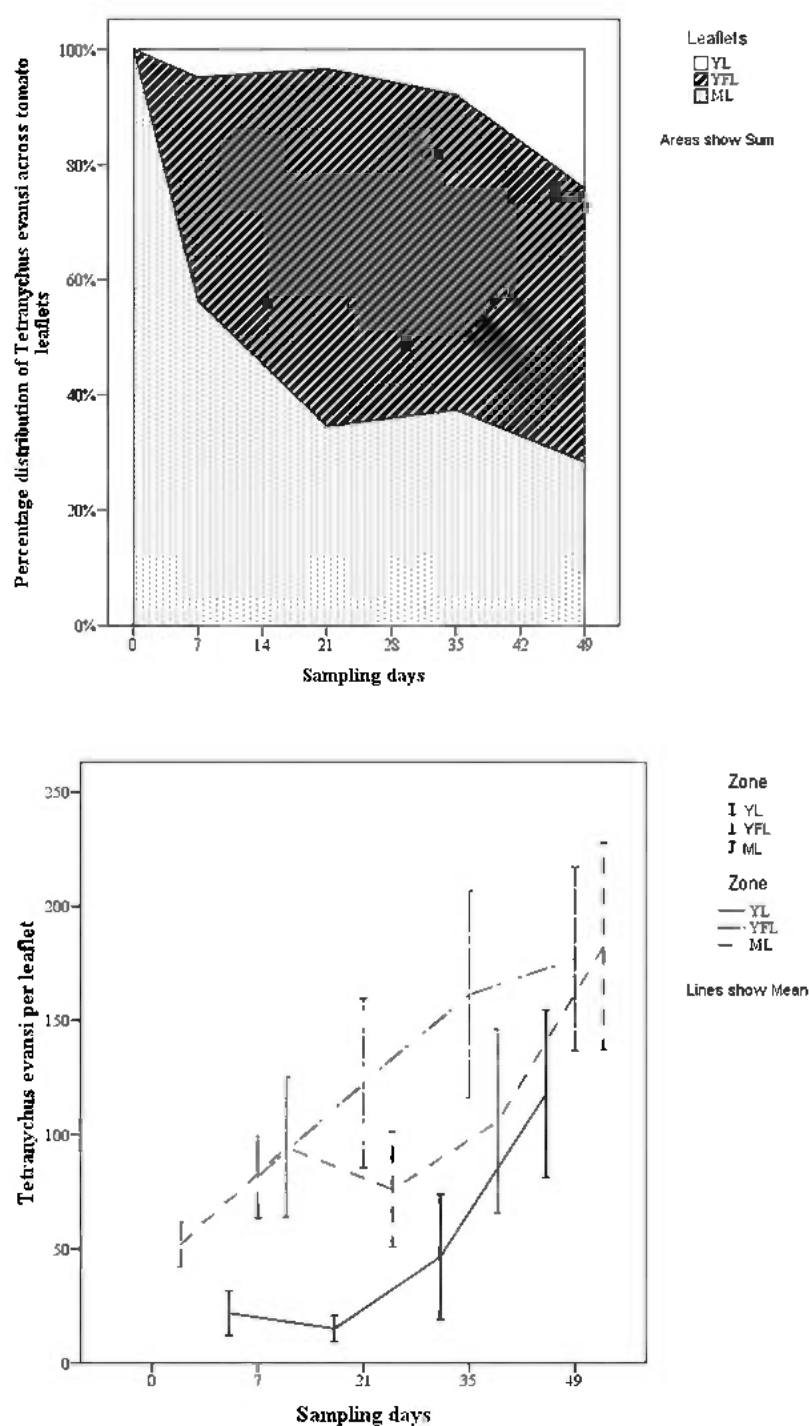
For the study, only YFLs (sampling unit) per plant was randomly selected for inspection whenever sampling was due. Since the number of YFL increased with time, β changed with time. Sampling for *T. evansi* was undertaken fortnightly. As a consequence, it became necessary to calculate β for each fortnight period of tomato production, meaning four values of β were calculated, one for each fortnight period during which destructive sampling was undertaken.

3. Results

3.1. Within-plant distribution of *T. evansi*

Over the course of the 49-day experimental period, there was an increase in the number of *T. evansi* mobile forms infesting the tomato plants. At the end of the experimental period, from the initial 30 mites per plant, the population of *T. evansi* mobile forms, had increased 10-fold (287 mites). Among the three canopy strata, at the end of the experimental period, the population densities of *T. evansi* exhibited differences with YFL having the highest population of red spider mites per plant ($n = 118$, $m = 134.55$, $SEM = 11.3$) followed by ML ($n = 158$, $m = 90.9$, $SEM = 8.1$). On the other hand, YL had the least population of red spider mites per plant ($n = 65$, $m = 61.9$, $SEM = 10.7$). There were significant differences in *T. evansi* population among the different canopy strata, Welch's $F(2,180.143) = 10.971$, $p < .0005$. Games-Howell post hoc analysis showed that the increase in *T. evansi* population from ML to YFL [43.665, 95% CI (10.87 to 76.46)], and the decrease in *T. evansi* population from YFL to YL [-72.643, 95% CI (-109.44 to 35.84)] were statistically significant ($p = .005$ and $< .0005$, respectively).

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Figure 1. Patterns of within-plant distribution of *T. evansi* in the tomato canopy strata throughout the growing season

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Regarding the different sampling dates, there were significant differences in *T. evansi* populations, Welch's $F(4,162.808) = 13.585, p < .0005$. The mite population increased from day 7 (77.17 ± 9.84) to day 21 (84.22 ± 12.18), day 35 (115.97 ± 15.93), day 49 (159.09 ± 14.62), in that order. Putting tomato canopy strata in perspective, at the onset of sampling (7 days post-inoculation), the concentration of spider mites was evenly distributed between ML and YFL (Figure 1). However, in the subsequent sampling days, YFL had the most consistent and highest proportion of the entire spider mite population. Also, upon carrying out regression analysis for the average number of *T. evansi* on YFL against the entire number of *T. evansi* on each plant for each of the 4 sampling dates,

there was a significant linear relationship (Table 1). Consequently, YFL appears as the most ideal part of the tomato plant to sample in order to assess *T. evansi* population.

Table 1: Regression equations for forecasting the number of *T. evansi* on a tomato plant by use of mean number of *T. evansi* on YFL stratum for plants of ages 3 to 9 weeks

Days post-inoculation	Intercept $\pm$ SE	Slope $\pm$ SE	Slope P value	R ²
7	163.54 $\pm$ 101.9	1.56 $\pm$ 0.25	<0.001	0.465
21	440.44 $\pm$ 181.7	1.85 $\pm$ 0.26	<0.001	0.532
35	2448.04 $\pm$ 940.5	1.36 $\pm$ 0.23	<0.001	0.438
49	8081.67 $\pm$ 2954.9	1.38 $\pm$ 0.25	<0.001	0.39

3.2. Development of binomial sequential sampling plan

Visual inspection of a scatterplot of variance against *T. evansi* mean showed a linear relationship between the variables (Figure 2). Also, there was normality and homoscedasticity of the residuals. The prediction equation for the association between the average number of *T. evansi* per YFL and the variance (Equation d), as projected by Taylor's power law, was statistically significant, $F(1,216) = 213.552$, $p < 0.0005$, and accounted for 49.7% of the variation in variance with adjusted $R^2 = 49.5\%$ - which is a large size effect [22]

$$\ln S^2 = 1.033 + 1.493 \ln m \quad (R^2 = 0.497) \quad (d)$$

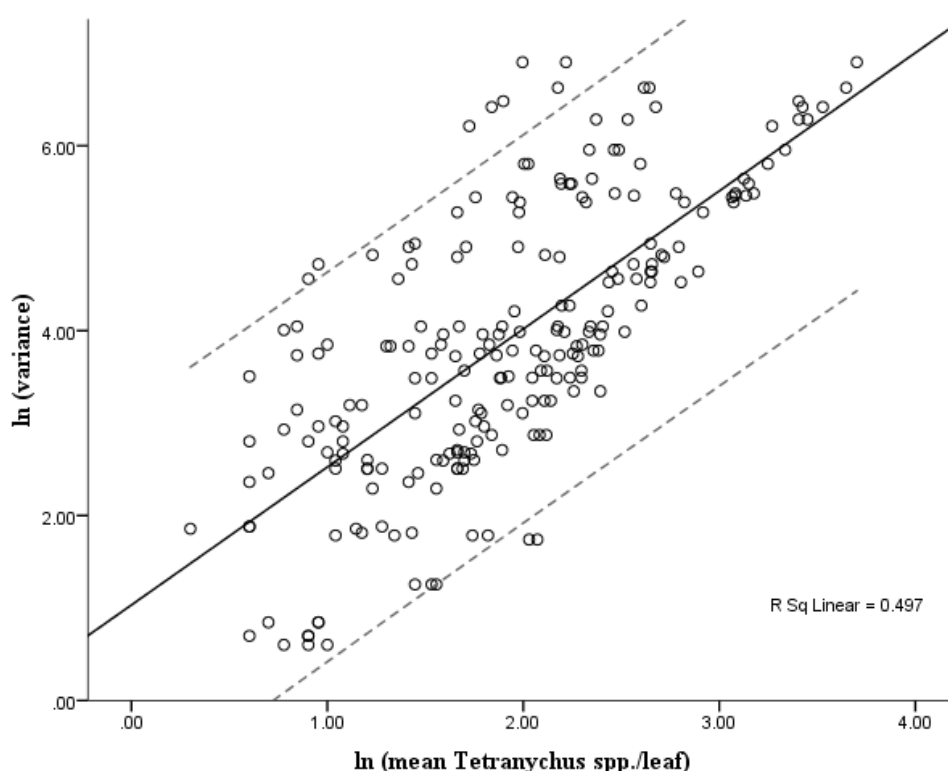


Figure 2. Association between the mean number of *T. evansi* and the variance on tomato leaves

On account of the high coefficient of determination ($R^2 = 0.497$), Taylor's power law effectively details the distribution of *T. evansi* on YFL. In addition, the slope was >1 . By implication this means that *T. evansi* populations enjoy an aggregated distribution.

Since the mean-variance association of *T. evansi* was expressed by Taylor's power law, the model was utilized to describe the distribution of *T. evansi* on tomato, and using values derived from the study ($a = 1.033$; $b = 1.493$).

Assessing the fitness of the binomial model through regression analysis, where the left and right-hand sides of equation b were compared against each other, there was a statistically significant association between the two sides, and for all the three tally thresholds (Table 2). Because the tally threshold of 5 mites per YFL produced the highest R^2 value, 5 mites per YFL was selected as the preferred tally threshold for *T. evansi*.

Table 2: Regression equations showing fitness of binomial model at different tally threshold

Tally threshold	Intercept $\pm$ SE	Slope $\pm$ SE	Slope P value	R^2
5	3.77 ± 0.11	1.52 ± 0.32	<0.0005	0.153
10	3.85 ± 0.10	1.18 ± 0.30	<0.0005	0.149
20	3.92 ± 0.09	0.88 ± 0.28	<0.0005	0.141

Running Cochran's Q test [23], the proportion of YFLs infested with *T. evansi*, at different time points, was found to be statistically significant, $\chi^2(3) = 38.458$, $p < .0005$. Also, significant effects were noted on account of variety ($F = 4.1$; $df = 2, 145$; $P = 0.019$) and inoculation rate ($t(144) = -3.061$, $P = 0.003$).

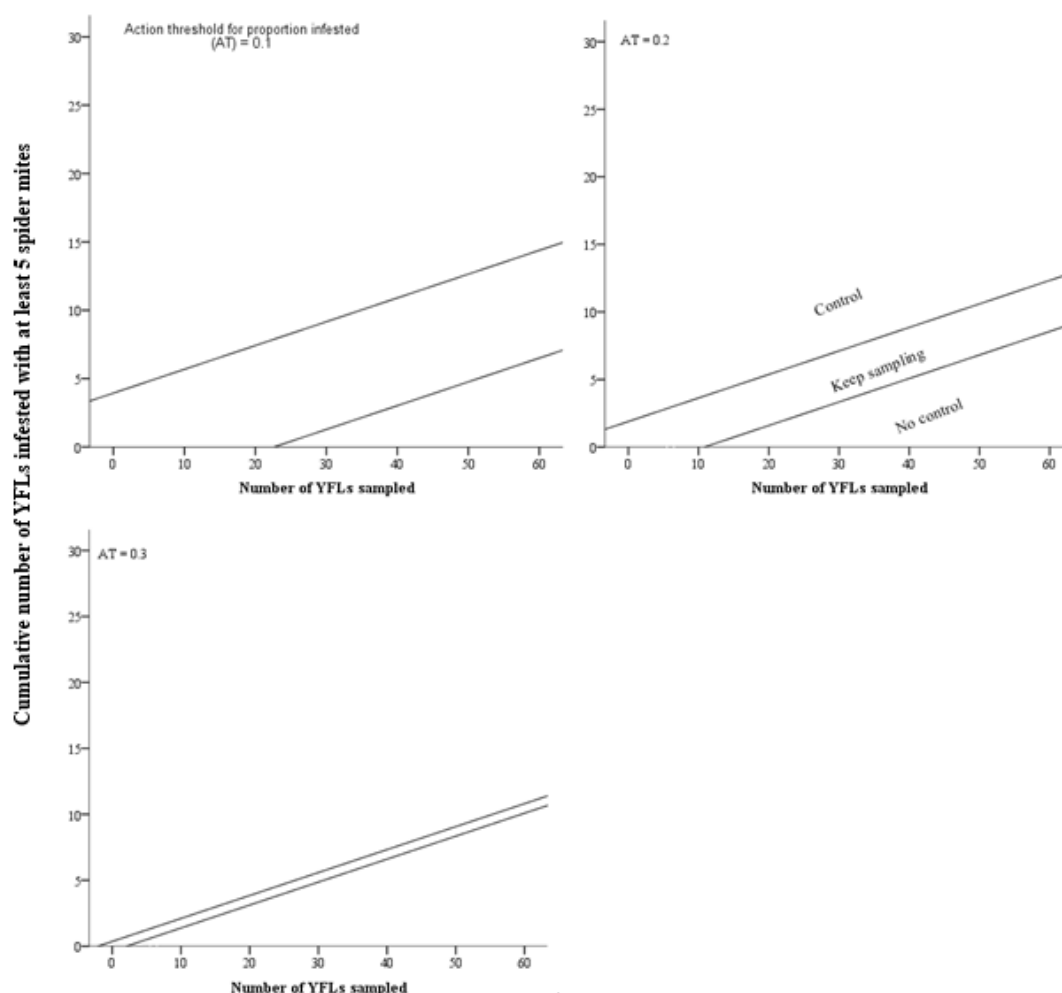


Figure 3. Decision boundary lines for the sequential sampling plan for spider mites on tomato based on three action thresholds (AT) of 0.1, 0.2, and 0.3 proportion of tomato infested with ≥ 5 spider mites and 0.05 α and β error parameters

For the three AT of 0.1, 0.2, and 0.3, the binomial sequential sampling plans are shown in Figure 3. While sampling, if the aggregate sum of infested sampling units rises beyond the upper boundary

line, the batch that has been sampled is regarded to be greater than the threshold, leading to a decision to treat. As well, if the aggregate sum of infested sampling units falls below the lower boundary line, the sampled batch is considered to be lower than the threshold, leading to a decision not to treat and to sample on another day.

3.3. Estimation of population density

In Table 3, the number of *T. evansi* on YFL sampled (as a fraction of the whole number on YFL) (β) and the number of *T. evansi* on the YFL (as a percentage of the entire number on all leaves) (γ) for plants of ages 7 – 49 days after inoculation are computed. The values of β and γ for plants of ages 7 – 49 days after inoculation are needed for the determination of *T. evansi* population density per square meter. Looking at β , its value was significantly influenced by sampling date ($F = 7.137$; $df = 3, 168$; $P < 0.001$). The type of variety, two-way interaction between season and time ($F = 1.112$; $df = 3, 168$; $P = 0.346$) and three-way interaction between season, time and variety ($F = 0.144$; $df = 6, 168$; $P = 0.99$) were not significant. The mean value of γ was 36%.

Table 3: Parameter estimates ($\pm$ SEM) of β (the proportion of total *T. evansi* on all middle zones) and γ (proportion of total *T. evansi* on all middle zones) for 3 – 9 wk.-old plants

Days post inoculation	Mean β	Mean γ
7	0.514 $\pm$ 0.07	0.635 $\pm$ 0.21
21	0.539 $\pm$ 0.065	0.433 $\pm$ 0.153
35	0.359 $\pm$ 0.063	0.293 $\pm$ 0.117
49	0.201 $\pm$ 0.031	0.074 $\pm$ 0.013

4. Discussion

Choosing an appropriate sampling unit is critical when developing a sampling plan. An analysis of within-plant distribution of *T. evansi* revealed that intermediate leaves (YFL) of ages 5 – 9 weeks sustained the apical and most steady proportion of the entire *T. evansi* population. Coupled with this, the YFL also held the highest mite density. Accordingly, non-destructive sampling plans designed for farmers should be anchored on counts of *T. evansi* in the YFL. Using the YFL as the sampling unit enhances efficiency and easiness of the sampling since the units to be sampled are comparable and readily recognized. Furthermore, the ability to detect *T. evansi* at depressed population densities is probable owing to the YFL being the most attacked.

The interaction between the location of the mite within the plant canopy and sampling date showed that the relative numbers of the mite in the three canopy strata changed over time. Immediately following inoculation, *T. evansi* was most abundant among the mature leaves, but as the season progressed, the mites became abundant among the YFL, while their numbers progressively increased among the YL. This phenomenon could be attributed to the fact that *T. evansi* exhibits gregarious behavior and only disperses to far-flung leaves when its density is high and after the depletion of food reserves in the more established leaves [3]. This phenomenon make it possible for targeted control of the spider mite (by targeting the areas of aggregation, 'high spots') within the plant, particularly at the onset of an infestation [3].

In this study, Taylor's regression model is used to assess the within-plant dispersion of *T. evansi* on tomato leaves - not only showing an aggregated distribution, but also high coefficients of determination. These aggregation indices provide primary information for developing sampling programs geared at estimating population density. For *T. evansi*, among the drivers for group-formation and living (aggregation) is the aspect of strengthened anti-predator functions [24, 25]. According to Dittmann and Schausberger (2017), anti-predator benefits of aggregation could arise from phenomena such as the dilution effect, encounter or avoidance effect, increased vigilance, cooperative defense, and predator confusion. The encounter or avoidance effect infers that an assemblage, though conspicuous, is often less likely noticed than individuals of the same group

scattered over the same area. On the other hand, the dilution effect posits that the presence of alternative targets in a group reduces the predation risk of individual members of a group [24]

Binomial sampling is constructed around the relationship between the sample mean and the proportion infested - as established through use of suitable tally thresholds (TT) [21, 26]. In the present study, a tally threshold of 5 *T. evansi* per YFL provided a good description of the association between the mean of mites and the proportion of infested leaves. By implication, this means, in instances where *T. evansi* population density is low, moderate or high, using a TT of 5 should be sufficient.

The boundary lines provide for three decision choices for each AT and the cumulative number of YFLs (infested with at least 20 *T. evansi*) needed to decide being based on the AT for each sampling plan. For the 10% AT, sampling at least 23 YFLs will be required before arriving at a decision (lower bound intersection) (Figure 3). For the 20 and 30% ATs the minimum numbers of YFLs are 11 and 3, respectively (Figure 3). In instances where a pest control decision cannot be made (the cumulative number of infested sampling units indefinitely falls between the lower decision line and the upper line), sampling should be deferred to a later time. According to Cocco et al. (2015), the resampling period should be informed by the projected pest population growth, and when the crop is due for harvesting.

Timing of the sampling is important and it ought not to commence prior to the pest population becoming active or after the damage associated with the pest becoming unacceptable. It would thus be convenient to link the binomial sequential sampling plan proposed in this study with other research, particularly on the degree-days required for *T. evansi* development.

The study further computes values of β (the number of *T. evansi* on sampled YFL as a percentage of the whole number on YFL) and γ (the number of *T. evansi* on the YFL as a percentage of the entire number on all leaves) for tomato plants of ages 3 – 9 weeks. These values are crucial when it comes to calculating *T. evansi* density per square meter in tomato plant. Knowing both the density of *T. evansi* per square meter and the size of the area infested (expressed in square meters) enables the determination of the number of *T. evansi* in the concerned area which is established by multiplying these two parameters. Noteworthy, it has been reported that there are several factors in the production of crops that could impact the value of β . These factors include irrigation, fertilization, interspecific competition, heating (cooling), lighting and plant density [16, 27, 28]. In addition, according to Wilson et al. (1983), from a pest management perspective, the appropriate reliability of population estimates is established by variation between the pest management decision threshold and the population density, and this falls within the domain of sequential sampling. In instances where population density is above or below the threshold, a small number of sample units are needed to establish the population lies on which side of the threshold. On the other hand, as the population density nears the threshold, the number of samples needed is greater [21].

5. Conclusions

Sequential sampling avails a mechanism for reducing and optimizing the sampling effort in instances where few samples are needed. Binomial sequential sampling, in particular, requires the slightest sampling effort since, unlike counting of pests on individual plants, evaluation of the presence or absence of a pest in a plant is less time-consuming. In the present study, an action threshold for *T. evansi* on tomato is premised on the proportion of tomato YFLs infested, instead of *T. evansi* counts. This bolsters the use of binomial data in the formulation of a sampling plan instead of data resulting from counts of an arthropod pest on individual plants. The binomial sequential sampling plan for *T. evansi* that has been developed in this study has the potential to significantly reduce the effort and time needed in the effective management of this pest. Notwithstanding, since the sampling plans are based on ATs and not exhaustively researched economic thresholds, there is room for improvement. Future research detailing the relationship between yield-loss and *T. evansi* damage on tomato would avail more precise thresholds for sampling plan development. Also, important for practitioners to customize the sampling plans developed here by considering costs

associated with pest management, an appropriate type I and II error rates, and best periods to truncate sampling in order to achieve the desired aims of the *T. evansi* management program.

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