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# Binomial Sampling Plan for Estimating *Tetranychus urticae* (Acari: Tetranychidae) Populations in Glasshouse Rose Grown by Arching Method

# 아치형 재배 시설장미에서 점박이응애의 이항표본조사법 개발

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**Abstract** - Infestations of two spotted spider mite (TSSM), *Tetranychus urticae* Koch, on glasshouse rose (*Rosa* sp.) grown by an arching method, were determined by counts of the number of TSSM per leaflet in Buyeo, Chungnam Province, for a 2-yr period. Binomial sampling plans were developed based on the relationship between mean density per leaflet ($m$), and proportion of leaflets infested with $< T$ mites ($P_T$), according to the empirical model $\ln(m) = \alpha + \beta \ln(-\ln(1-P_T))$. $T$ was defined as tally threshold, and set to 1, 3, 5, 7, and 9 mites per leaflet. Increasing sample size had little effects on the precision of the binomial sampling plan, regardless of tally threshold. However, the precision increased with higher tally thresholds. There was a negligible improvement in precision with $T > 7$ mites per leaflet. $T = 7$ was chosen as the best tally threshold for estimating densities of TSSM based on the precision of the model. Independent data set was used to evaluate the model. The binomial model with $T = 7$ provided reliable predictions of mean densities of TSSM observed on the commercial glasshouse roses.

**Key Words** - Acari, *Tetranychus urticae*, Two spotted spider mite, Binomial sampling, Tally threshold

**초 록** - 충남부여에 위치한 임업화훼단지내의 유리온실에서 아치형재배 (Arching method) 장미에 피해를 주는 점박이응애 (*Tetranychus urticae* Koch)의 밀도를 엽당 응애수로 조사하였다. 이항표본조사법은 엽당 점박이응애의 평균밀도 ($m$)와 점박이응애가 T개체보다 많이 존재하는 엽의 비율 ($P_T$)과의 관계를 기본으로 하며, T는 경험적 이항분포모형 ($\ln(m) = \alpha + \beta \ln(-\ln(1-P_T))$)에서의 tally threshold로서, 본 실험에서는 1, 3, 5, 7, 9를 사용하였다. 일반적으로 표본단위 수의 증가는 $T$와 상관없이 이항분포 모형의 정확도에 영향을 거의 주지 않게 된다. 본 실험에서는 상이한 $T$에 따라 이항분포모형의 정확도가 차이가 났으며, $T$가 증가할수록 정확도가 높아졌다. 본 실험결과 점박이응애의 밀도추정을 위한 이항분포모형의 정확도를 비교한 결과, $T = 7$인 경우가 최적의 tally threshold인 것으로 나타났다. 또한 이항표본조사법의 검정을 위하여, 동일한 포장의 독립적인 표본을 추출, 조사하였다. 본 실험결과 이항표본조사법을 이용한 상업적 유리온실의 아치형재배 장미해충인 점박이응애 평균밀도 추정에는 $T = 7$인 경우가 가장 적절할 것으로 사료된다.

**검색어** - 점박이응애, 이항표본조사법, 시설원예, 아치형 장미재배

The two spotted spider mite (TSSM), *Tetranychus urticae* Koch, is an economically important pest of greenhouse roses (Van de Vrie, 1985), which are the most important cut flower crop grown in Korea for the

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local and international markets. The traditional method of control has been to use miticides on a calendar basis with little regard for pest population levels. Integrated control using the predatory mite, *Phytoseiulus persimilis* Athias-Henriot, has now become a practical alternative in Korea, but major obstacle to its adoption is the lack of grower management expertise. Growers need a simple sampling system to time the release of predators and the application of miticides accurately (Hepworth and MacFarlance, 1992).

Over the past ten years, a major change in the production systems of rose as cut flowers has been initiated in Korea. At present, the most popular growing method is the arching method, originated in Japan (Ohkawa, 1992). The popularity of this method is due to its production of quality roses. This method is practiced in the rock wool media on raised bench (Boodley, 1998). To manage the timing of flower production, the shoot is bent down instead of pinching the terminal growing point of shoot. As a result, the rose plants arch over the raised bench. Arching the shoots is continued to cause the development of a large amount of leaf area that carries on the photosynthesis for quality rose production. High photosynthetic rate can be maintained throughout the growing seasons, because a large amount of leaf areas is existed continuously. In addition, arching method does not require the pruning, bush cut, and supporting wires which are crucial steps in the pinching method (Boodley, 1998).

Change in crop production systems profoundly influences ecology and dynamics of insect pests. Recognizing the importance of change of crop production system and understanding its role is essential in the development and implication of effective pest management. TSSM populations on dense foliage, continuously existing during the seasons, in the arching method can serve as an infestation source, because the plants are never pruned or cut backed. Thus, it may be necessary to monitor the TSSM population frequently in the arching method. The dense foliage in the arching method, moreover, can play a role of the protective shield for TSSM against miticides.

Sampling programs for TSSM can help to determine whether sprays or predator releases are needed to prevent cosmetic injury, loss in yield, or both in greenhouse roses (Sanderson and Zhang, 1995). A major impediment to establishing an IPM program for TSSM is that a sampling program was never developed for this pest on greenhouse rose grown by the arching method in Korea. Most sampling programs for TSSM were developed on the greenhouse roses grown by traditional cultivation such as the pinching method (So, 1991; Sanderson and Zhang, 1995). The objectives of this study were to develop and evaluate the binomial sampling plans for estimating population density of TSSM on glasshouse rose grown by the arching method.

# Materials and Methods

## Sampling

TSSM populations were sampled weekly or biweekly from commercial rose glasshouses at Buyeo, Chungnam Province from June to January during 1997 and 1998. Rose (*Rosa* sp. 'Rote' ) plants have been grown in the rock wool media by the arching method for over 4 yr.

Ths study was done in two identical glasshouses, each of which was 2,500 m$^2$ in area and contained twenty beds of roses. The beds were each 81.6 m$^2$ in area (1.2 × 68 m) with 0.8 m height and spaced 1.2 m apart. The rose plants were placed at a spacing of 0.2 m × 0.2 m on the bed. Each bed contained 4,800 plants, arranged in 4 rows of 1,200 plants. Miticides were applied regularly to roses for the control of TSSM. Temperature in the glasshouses fluctuated between 18~38°C. Humidity and light levels were not controlled, but were similar in both glasshouses.

On each sampling date, 25 rose plants were randomly selected from 5 beds. For each plant, 3 five-leaflet leave were arbitrary selected from each of two canopy strata: the lower canopy ($\approx$0.3 m from the ground) and upper canopy ($\approx$0.6 m from the ground). Thus, a total of 75 five-leaflet leaves were sampled per plant stratum on each sampling date. All sample leaflets were removed from the plants and immediately sprayed with Spray Adhesive (3M®, USA) to stop all TSSM movement. The samples from each stratum in each bed were kept separately in a ice chest and brought back to the laboratory for examination. Adult and immature (includ-ing egg) mites on five-leaflet leaf were counted under a dissecting microscope. Because one-leaflet sample unit was more efficient than either the 3- or and 5-leaflet units (Park *et al.*, 1998), this unit was used to measure TSSM density for the reminder of the study.

## Development of Binomial Sampling Plan.

Because the size of TSSM egg was too small to count *in situ*, only the data for adult and immature mites were used for the analysis. The empirical model of Kono and Sugino (1958) was used to determine the relationship between mean density ($m$) and the proportion of sampling unit infested with at least $T$ mites ($P_T$):

$$\ln(m) = \alpha+\beta\ln(-\ln(1-P_T)) \tag{1}$$

where $\alpha$ and $\beta$ are parameters estimated by the general linear regression model procedure (PROC GLM, SAS Institute, 1995). The model parameters were estimated for the different tally threshold ($T$) of 1, 2, 3, 5, 7 and 9 mites per leaflet.

Calculation of a valid variance for a population prediction is necessary for evaluating precision of the binomial sampling programs (Binns and Bostanian, 1990). The estimated variance of ln $m$ from equation 1 is comprised of mainly three sources of error: a variance component resulting from the estimation of the parameter of $\alpha$ $\beta$ and ($c1$), a component originating from the estimation of $P_T$ ($c2$) and a component from the biological error. Schaalje *et al.* (1991) proposed that the biological variance can be estimated by removing a sampling error component ($c3$) from the residual mean square error of the regression ($c4$). Thus total variance of ln $m$ can be estimated from the equation (2).

$$\begin{aligned} \mathrm{var}(\ln m) &= (c1+c2+(c4-c3)), \\ c1 &= (\beta^2 P_T)/\,n(1-P_T)\ln(1-P_T)^2 \\ c2 &= \frac{MSE}{N} + (\ln(-\ln(1-P_T))-\overline{P})^2 s^2{}_\beta \\ c3 &= \exp(\ln a+(b-2)(\alpha+\beta\ln(-\ln(1-P_T))))\,/n \\ c4 &= MSE \end{aligned} \tag{2}$$

where $MSE$ = the mean square error from equation 2, $N$ = the number of data points in the regression used to estimate $\alpha$ and $\beta$ from equation 1, $\overline{P}$ = the average value of $(-\ln(1-P_T))$ used in the regression, $s^2{}_\beta$ = the sample estimate of variance of $\beta$, and $n$ = the number of samples taken from a population. The parameter $a$ and $b$ of Taylor's power law (Taylor, 1961) were taken from Park *et al.* (1998) with $a = 7.85$ and $b = 1.70$.

The precision of the binomial sampling programs was evaluated and compared at different tally thresholds. Defining precision ($d$) as the standard error of mean, $d = \sqrt{\frac{s^2}{n}}$, and substituting equation 2 for $\frac{s^2}{n}$ gives (Nachman, 1984):

$$d = \sqrt{(c1+c2+(c4-c3))} \tag{3}$$

### Validation of Sampling Plans.

The reliability of the binomial sampling plan was evaluated using a data set not used in developing the sampling plan. Glasshouse condition and agronomic practice were same to the glasshouses used for the development of binomial samplings described above. Sampling methodology followed the procedure described before, except that only forty sample units were collected from each canopy stratum. This glasshouse had been sampled 11 times.

Homogeneity of the slopes ($\beta$) from the predicted models and the validation data set was tested with the analysis of covariance (ANCOVA) (Sokal and Rohlf, 1981). Equality of intercepts ($\alpha$) was determined by continuation of ANCOVA. Bias of the predictions for $T$ = 1, 3, 7 and 9 were calculated by $\bar{e} = \Sigma e/n$ where $e$ = (predicted ln $m$ − observed ln $m$) (Tonhasca *et al.*, 1994). The significance of bias was estimated with $W$, which is an approximation of the standard normal distribution. The statistics $W$ was calculated as $\bar{e}\sqrt{n/s}$, which $s$ is the standard deviation of ln $m$ and the critical value was obtained from a $t$ table with $n-2$ degrees of freedom (Power, 1993; Tonhasca *et al.*, 1994).

## Results and Discussion

A total of 36 separate population estimates were collected during this study. However, after elimination of $P_T$ values of 0 and 1, only 30 to 36 of the estimates were used for the development of the binomial sampling plan, depending on the tally thresholds (Table 1). The overall proportions of infested sample unit with TSSM were 0.22 and 0.26 from the lower and upper canopy strata, respectively. These low infested-proportions were expected, because miticides were frequently applied on a calendar basis for the control of TSSM during this study. The data from two plant canopy strata were combined and calculated the infested proportions, because spatial distribution patterns (Park *et al.*, 1998) and infestation rates were similar to each other. Thus, the binomial sampling plan can be equally applicable to the lower and upper plant canopy strata.

The relationship between the proportion of infestation for $T$ = 1, 3, 7 and 9 and observed means for $m > 6.4$ are presented in Fig. 1. The relationship at $T$ = 5 was not included, because the result was similar to $T$ = 3. The

Table 1. Parameters of an empirical binomial model (equation 1) relating mean two-spotted mites per leaf to the proportion of leaflets infested with at least *T* mites

| *T* | $\alpha$ | $\beta$ | $r^2$ | N | P | $s^2_\beta$ | MSE |
|---|---|---|---|---|---|---|---|
| 1 | 3.1167 | 1.3943 | 0.69 | 36 | −2.4426 | 0.0259 | 0.6819 |
| 2 | 3.1606 | 1.2411 | 0.74 | 35 | −2.6789 | 0.0162 | 0.4236 |
| 3 | 3.1671 | 1.1387 | 0.79 | 34 | −2.8379 | 0.0109 | 0.2844 |
| 5 | 3.4308 | 1.9208 | 0.80 | 34 | −3.1186 | 0.0099 | 0.2700 |
| 7 | 3.5391 | 1.0836 | 0.86 | 31 | −3.1550 | 0.0068 | 0.1518 |
| 9 | 3.7961 | 1.1109 | 0.84 | 30 | −3.2596 | 0.0082 | 0.1565 |

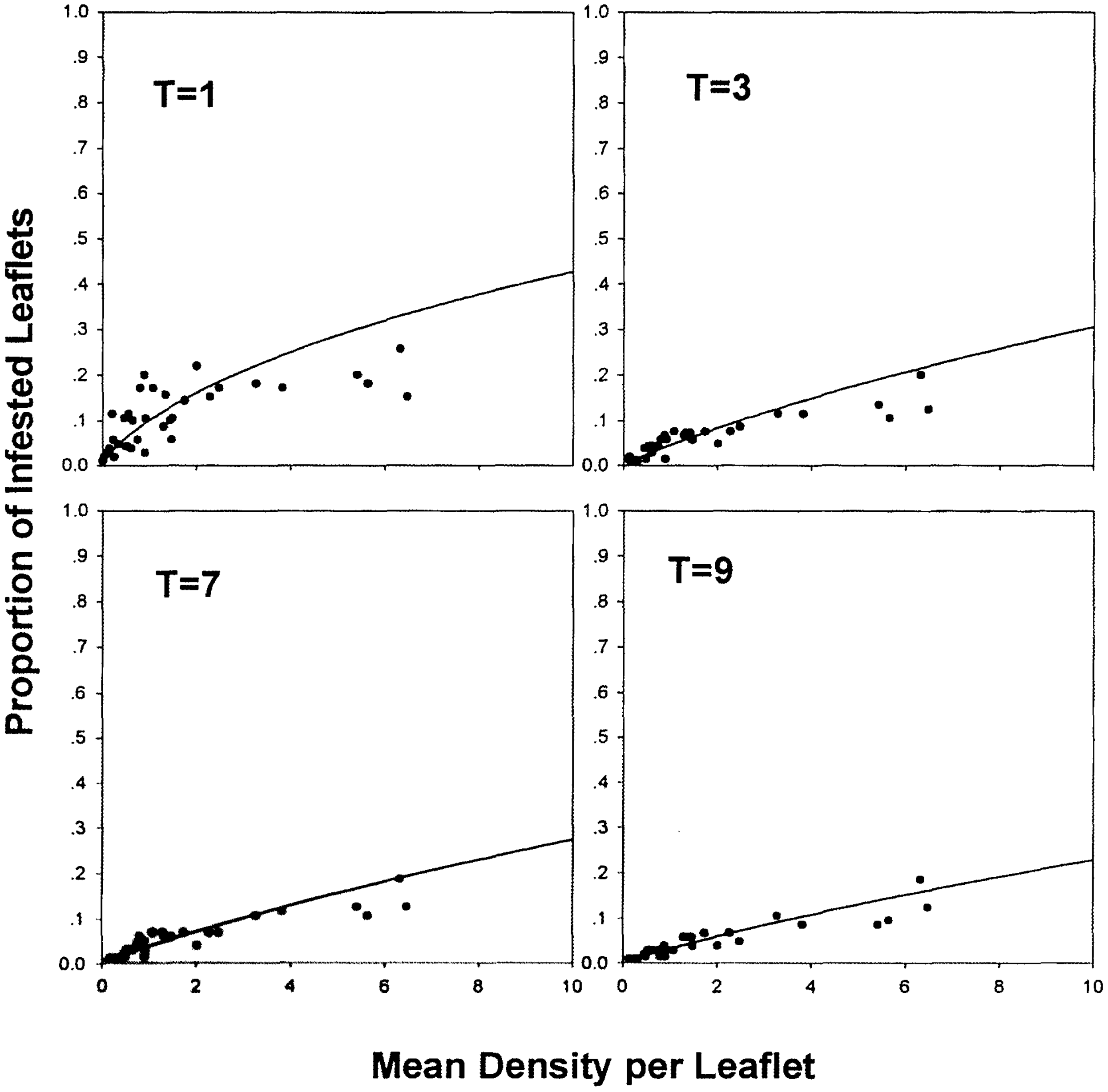

Fig. 1. Relationship between the proportion of sample unit infested and the mean number of mites with *T* = 1, 3, 7 and 9 as estimated by Kono and Suguino model.

empirical binomial model was statistically significant for all the tally thresholds with $r^2$ values ranging from 0.69 to 0.86 (Table 1). The highest $r^2$ value was observed at $T = 7$ with 0.86. Examination of the residual error from the regression (MSE) indicated that model fits improved up to $T = 7$ and declined slightly with $T > 7$. This implies that the binomial model fits best at $T = 7$.

The effects of the sample size and tally threshold on the estimation of mean density were examined by determining precision as a function of the proportion infested and number of sample unit examined (Fig. 2). Sample size ($n$) had little effect on sampling precision, regardless of tally thresholds (Jones 1994; Naranjo *et al.*, 1996). In contrast to sample size, increasing $T$ had a dramatic effect on the precision of the binomial sampling plans (Fig. 2). The precision was improved up to $T = 7$ and then declined slightly with $T > 7$. However, the precision never exceeded ≈0.41 at $T = 7$. This result was primarily from the relative stability of in MSE at $T = 7$ (Table 1), and the fact that MSE is the largest variance component (Nyrop and Binns, 1991).

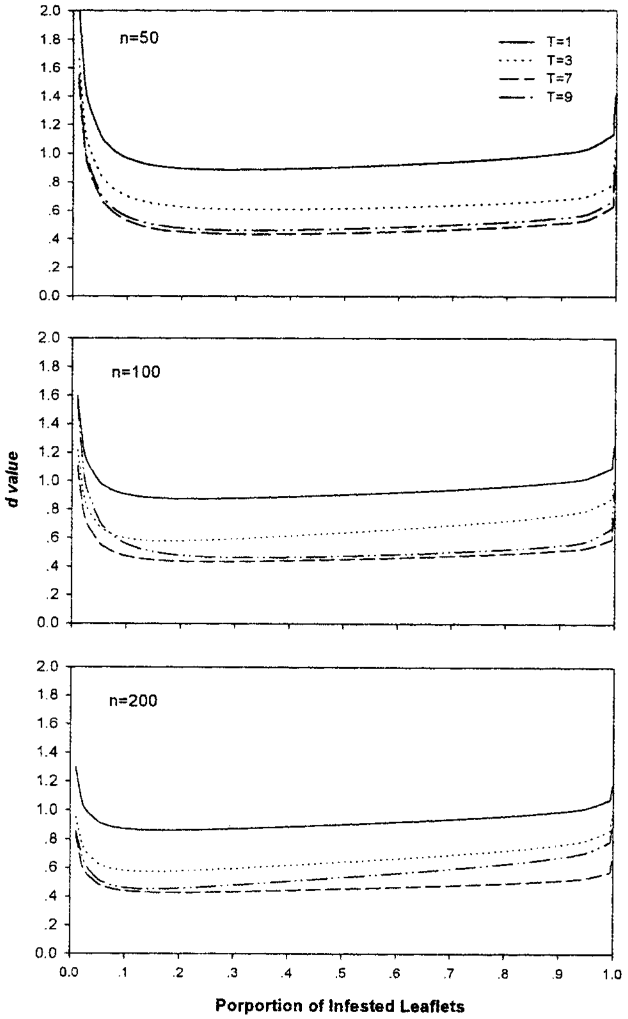

Fig. 2. The sampling precision ($d$) expressed as a function of proportion of infested at the sample size of 50, 100 and 200.

The proper selection of $T$ can reduce the variability and bias of the estimated mean and provide for acceptable error rates and average sample sizes when using sequential classification (Nyrop and Binns, 1991). In this study, regardless of tally threshold or sample size, it was not possible to obtain the level of precision $d = 0.25$ suggested by Southwood (1978) for damage level assessment in a pest management program. These relatively low precision levels reflect the high variability of binomial sampling plans when compared to absolute counts (Nyrop and Binns, 1991). Other methods proposed to estimate the variance of the binomial empirical model would result in different values, as they place different weight on the individual variance components (see Jones, 1994). Regardless of the methods, the binomial sampling plans developed in this study could not provide precision of mean density better than 0.40 at different tally thresholds or sample sizes. Thus, the reliability of the variance estimation methods needs corroboration from field data (Tonhasca *et al.*, 1994).

Based on the above analysis, a binomial model at $T = 7$ appears to be the best for estimation of mean densities of TSSM in glasshouse roses. In general, the variance in estimating mean density declined with increasing $T$ up to a certain point (Binns *et al.*, 1992; Feng *et al.*, 1993; Naranjo *et al.*, 1996). The particular values of $T$ that was best varied in relation to the sample size examined, the species under study, and methods used to evaluate the model performance. Naranjo *et al.* (1996) reported that the binomial sampling plan with $T = 3$ and a fixed sample size of 30 were an effective replacement of enumerative counts for adult *Bemisia tabaci* (Gennadius) on cotton.

Estimating the mean with high tally thresholds is less practical than the simple detecting the presence of TSSM, because sampling speed would have to be reduced for a more careful examination of leaves. However, Nyrop and Binns (1991) found that the increased time was not as significant as expected for sampling of the European red mite at various tally thresholds; the sampling time only doubled when the tally threshold went from 1 to 8.

Table 2. Statistics of the empirical model (equation 1) for the validation data set for tally thresholds ($T$), ANCOVA test for comparisons with predicted $\alpha$ and $\beta$ (Table 1), bias ($\bar{e}$) and corresponding test of significance ($W$)

| $T$ | $\alpha$ | $\beta$ | $\bar{e}$ | $df^a$ | $\lvert W \rvert$ |
|---|---|---|---|---|---|
| 1 | 2.3157* | 0.8319* | −0.4728 | 48 | 2.9970* |
| 3 | 3.3002* | 1.0719 | −0.3121 | 46 | 2.1460* |
| 7 | 3.8035 | 1.0949 | −0.2311 | 42 | 1.6088 |
| 9 | 3.8085 | 1.0327* | −0.2441 | 40 | 1.7131 |

$^a$ $n_1+n_2-4$
* $P < 0.05$

Mean density levels of TSSM in a validation data set were similar to the data sets used for developing the binomial sampling plans, and ranged from 0.70 to 6.88 per leaflet. ANCOVA test demonstrated that the models were appropriate only for $T=7$ and 9 to describe the relationship of the proportion of infested and the corresponding density (Table 2). The slopes and intercepts at these tally thresholds were not significantly different between the predicted models and validation data set ($P > 0.05$). However, either slopes or intercepts from the predicted models with $T=1$ and 3 were significantly different from the validation data set ($P < 0.05$). These results suggested that there is a high degree of relationship between the predicted models and validation data set at $T=7$ and 9. The result of $W$ statistic indicated significant underestimation of the expected mean values for $T=1$ and 3 (Table 2). The model $T=7$ was the less biased and more efficient than the model $T=9$. Thus, the empirical model with $T=7$ was relatively robust in relation to prediction of mean density of TSSM in glasshouse roses.

The binomial sampling plans presented here should greatly enhance the efficiency of monitoring population of TSSM in glasshouse roses. The binomial model based on $T=7$ has a significant advantage over the presence-absence approach ($T=1$). Using $T=7$ increased precision roughly 2.5-fold compared to $T=1$. However, the precision level is not high enough for the pest management decision makings suggested by Southwood (1978). The performance of the empirical model depends on the suitability of the least-square linear regression for describing data. Ideally, values of the dependent and independent variables should be evenly distributed along the range of data (Tonhasca *et al.*, 1994). The low precision levels observed in this study may be, due in part, from the limited ranges of infested proportions. Maximum proportions of infested in this study were restricted within a narrow range; 0.15 to 0.35 depending on the tally thresholds. However, the binomial sampling plan is promising for glasshouse roses because TSSM usually infests the commercial glasshouses with very low level and develop damaging populations very rapidly; constant monitoring of TSSM density is required. Additional studies will be necessary to evaluate the tradeoff between the relatively low precision and the considerable reduced effort of the sampling schemes proposed here (Tonhasca *et al.*, 1994).

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